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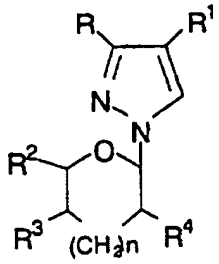
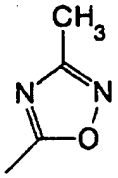
(57) Abstract

Pyrazole-related derivatives having general formula (I) are described, wherein R is COOH, COOR', and (III) and R' is a C_1-C_5 alkyl radical, R^1 is I, Br, Cl, F, CF_3, CN, SCN, CHO, $CH{=}CH_2$, CH=CH Br, $C{\equiv}C\text{-}Si(CH_3)_3$, CH=CH $COOCH_2CH_3$, C≡CH, $CH_2{=}CH$ NO_2, n is 0 or 1, R^2 is H, CH_2OH, CH_2 OCOPh, CH_2O $Si(CH_3)_2C(CH_3)_3$, CH_2OPO_3 Na_2, $CH_2OPO[OCH_2O\text{-}COC(CH_3)_3]_2$, $CH_2OPO[O\ CH_2\ CH_2\ SCOC(CH_3)_3]_2$, R^3 and R^4 equal or different from each other are H, OH, CH_2 OCOPh, CH_2O $Si(CH_3)_2C(CH_3)_3$, or they form together an isopropylidene group, provided that when $R^2 = R^3 = R^4 = H$ n is always 1, and with the exclusion of the compound having R=$COOCH_3$, R^1 = I, n=O, $R^3 = CH_2$ OCOPh, $R^3 = R^4$=OCOPh. These compounds are endowed with antitumor and are able to enhance antiviral activity of known antiviral agents. The procedures for preparation of compounds of formula (I) and of pharmaceutical formulations containing them are also reported.



(I)



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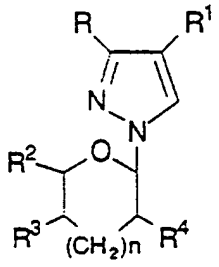
PYRAZOLE-RELATED DERIVATIVES ENDOWED WITH ANTITUMOR AND ANTIVIRAL ACTIVITIES, PROCEDURES FOR THEIR PREPARATION, PHARMACEUTICAL FORMULATIONS CONTAINING THEM.

FIELD OF THE INVENTION

This invention refers to new pyrazole-related derivatives having general formula (I)



(I)

wherein R is COOH or COOR',

and R' is a C_1 -C_5 alkyl radical

R^1 is I, Br, Cl, F, CF_3, CN, SCN, CHO, $CH=CH_2$, CH=CH Br, C≡C-Si $(CH_3)_3$, CH=CH $COOCH_2CH_3$, C≡CH, CH_2=CH NO_2,

n is 0 or 1,

R^2 is H, CH_2OH, CH_2 OCOPh, CH_2O $Si(CH_3)_2C(CH_3)_3$, $CH_2OPO_3Na_2$, $CH_2OPO[OCH_2O-COC(CH_3)_3]_2$, $CH_2OPO[O\ CH_2\ CH_2\ SCOC(CH_3)_3]_2$,

R^3 and R^4 equal or different from each other are H, OH, CH_2 OCOPh, CH_2O $Si(CH_3)_2C(CH_3)_3$, or they form together an isopropylidene group,

provided that when $R^2 = R^3 = R^4$ = H n is always 1.

and with the exclusion of the compound having R=$COOCH_3$, R^1 =I, n=0, $R^3 = CH_2$ OCOPh, $R^3 = R^4$=OCOPh

This invention refers also to pharmaceutically acceptable salts and soluble derivatives of compounds of formula (I), to processes for their preparation and to their use in pharmaceutical formulations for the treatment of tumors and viral infections, with particular regard to infections carried out by human immunodeficiency viruses (HIV) and herpesviruses.

PRIOR ART

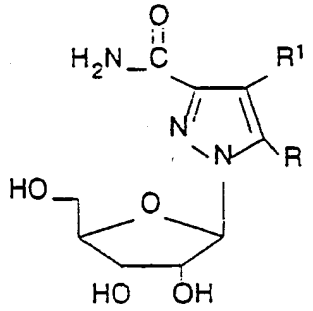
The most important goal in antitumor chemotherapy is the development of selective drugs that might be able to inhibit the multiplication of cancer cells without interfering with the growth of normal cells.

Ribavirin (1–β–D–ribofuranosyl–1,2,4–triazole–3–carboxamide), a drug that has been described 20 years ago as a broad–spectrum antiviral agent and which also displays antitumor activity in mice, has attracted considerable attention because of its peculiar mechanism of action, being an inhibitor of inosine monophosphate dehydrogenase (IMPDH). For this reason, a number of structural analogs of ribavirin have been developed. Among them are tiazofurin (2–β–D–ribofuranosyl–tiazole–3–carboxamide), selenazofurin (2–β–D–ribofuranosyl–selenazole–4–carboxamide), ribamidin (1–β–D–ribofuranosyl–1,2,4–triazole–3–carboxamidine), FICAR (5–fluoro–1–β–D–ribofuranosyl–imidazole–4–carboxamide) and EICAR (5–ethynyl–1–β–D–ribofuranosyl–imidazole–4–carboxamide).

Besides their activity against a wide variety of human tumours, the above cited derivatives, and in particular ribavirin, are active against orthomyxoviruses (Influenza A and B), paramyxoviruses (parainfluenza, measles, respiratory syncytial virus), arenaviruses and bunyaviruses. They are also active against pox, picorna–, toga and reoviruses.

Ribavirin has been reported to prolonge the survival of monkeys infected with Lassa fever virus; it is used for the treatment of infections caused by respiratory syncytial virus and, so far, is the sole drug endowed with some efficacy in the treatment of viral haemorragic fevers in man.

Recently, Manfredini *et al.* (J. Med. Chem., 1992, *35*, 917–924) have reported some 1–β–D–ribofuranosyl–1,2–diazole–derivatives structurally related to ribavirin, of general formula (II)



wherein:

R may be H or CH_3 and R^1 may be Br, I, NO_2, NH_2 or H.

These compounds have been shown to possess selective cytostatic activity against lymphoblastoid T cells, but their antiproliferative activity was not potent enough to be therapeutically usefull.

Recent studies on the mechanism of action of ribavirin, tiazofurin and selenazofurin, which focus on the crucial role of the primary amide at C-3, have led to the conclusion that the biological activity of these drugs is related to steric and hydrogen-bonding requirements at this position. These compounds are therefore believed to be structurally related to inosine monophosphate (IMP), a prerequisite for their binding to the IMPDH [Manfredini *et al.* (J. Medical Chem., 1992, *35*, 917-924) and Gabrielsen *et al.* (J. Medical Chem., 1992, *35*, 3231-3238)].

The need for new drugs that might be useful for antitumor and antiviral chemotherapy has led us to continue research in this field with the aim of discovering new active molecules.

SUMMARY OF THE INVENTION

This invention deals with new pyrazole-related derivatives of general formula (I) carrying, as reported above, a carboxylic acid, an ester moiety or an oxadiazole at the position C3 of the pyrazole heterocycle. It also deals with their pharmaceutically acceptable salts and soluble derivatives that could be useful for the antitumor and antiviral chemotherapy.

In particular, the compound that will be indicated as **2** proves more active, as an antiproliferative agent, than ribavirin and as active as selenazofurin. Furthermore, when compared to selenazofurin, **2** proves less cytotoxic against resting PBL (peripheral blood lymphocytes).

In addition, the present invention refers to processes for the preparation of compounds having general formula (I) and to their use in pharmaceutical formulations useful for the antitumor chemotherapy and for the treatment of viral infections such as those caused by human immunodeficiency viruses (HIV) and herpesviruses. In fact, compounds of formula (I) were able to enhance the antiviral activity of 2',3'-dideoxy-inosine (ddI) 2',3'-dideoxy-adenosine (ddA), 9-[2-(phosphono-methoxy)ethyl]-adenine (PMEA), 9-[2-(phosphono-methoxy)propyl]-guanine (PMPG), 9-[2-(phosphono-methoxy)propyl]-adenine (PMPA), acyclovir (ACG), 9-[2-(phosphono-methoxy)-ethyl]-guanine (PMEG) and adenine-arabinoside (araA).

Therefore, the present invention also refers to the use of compounds of formula (I) with the aim of potentiating the anti-HIV activity of ddI, ddA, PMEA, PMPG, PMPA and the antiherpes activity of ACG, PMEG and araA. It also refers to pharmaceutical formulations containing the compounds of formula (I) with at least one antiviral agent such as ddI, ddA, PMPG, PMPA for

the treatment of HIV infections or in combination with antiviral agents such as ACG, PMEG and araA useful for the treatment of herpetic infections also in AIDS patients.

Another fundamental feature of this invention is that it refers to new intermediates of the above preparation processes.

DETAILED DESCRIPTION OF THE INVENTION

Based on their substituents, the compounds of the present invention having general formula (I) can be described as follows (Bz indicates the benzoyl protecting group):

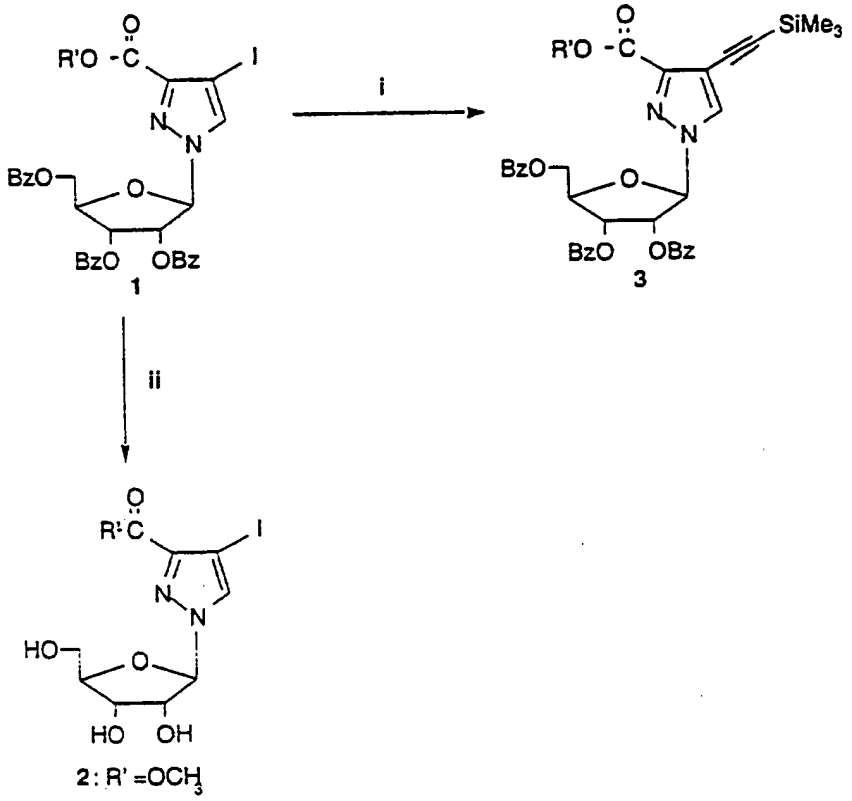
- Compound **2**: R = $COOCH_3$, R^1 = I, n = 0, R^2 = CH_2OH, R^3 = R^4 = OH;
- Compound **3**: R = $COOCH_3$, R^1 = $C{\equiv}CSi(CH_3)_3$, n = 0, R^2 = CH_2OBz, R^3 = R^4 = OBz;
- Compound **5**: R = $COOCH_2CH_3$, R^1 = CHO, n = 0, R^2 = CH_2OBz, R^3 = R^4 = OBz;
- Compound **6**: R = $COOCH_2CH_3$, R^1 = $CH(OH)CH_2NO_2$, n = 0, R^2 = CH_2OBz, R^3 = R^4 = OBz;
- Compound **7a**: R = $COOCH_2CH_3$, R^1 = $CH{=}CHCOOCH_2CH_3$, n = 0, R^2 = CH_2OBz, R^3 = R^4 = OBz;
- Compound **7b**: R = $COOCH_2CH_3$, R^1 = $CH{=}CH_2$, n = 0, R^2 = CH_2OBz, R^3 = R^4 = OBz;
- Compound **7c**: R = $COOCH_2CH_3$, R^1 = CH=CHBr, n = 0, R^2 = CH_2OBz, R^3 = R^4 = OBz;
- Compound **7d**: R = $COOCH_2CH_3$, R^1 = $CH{=}CHNO_2$, n = 0, R^2 = CH_2OBz, R^3 = R^4 = OBz;
- Compound **8**: R = $COOCH_2CH_3$, R^1 = I;
- Compound **9**: R = $COOCH_2CH_3$, R^1 = I, n = 1, R^2 = R^3 = R^4 = H;
- Compound **10**: R = $COOCH_2CH_3$, R^1 = CHO, n = 1, R^2 = R^3 = R^4 = H;
- Compound **11**: R = $COOCH_2CH_3$, R^1 = $C{\equiv}CSi(CH_3)_3$, n = 1, R^2 = R^3 = R^4 = H;
- Compound **12**: R = $COOCH_2CH_3$, R^1 = $C{\equiv}CH$, n = 1, R^2 = R^3 = R^4 = H;
- Compound **13**: R = $COOCH_2CH_3$, R^1 = $CH(OH)CH_2NO_2$, n = 1, R^2 = R^3 = R^4 = H;
- Compound **14a**: R = $COOCH_2CH_3$, R^1 = $CH{=}CHNO_2$, n = 1, R^2 = R^3 = R^4 = H;
- Compound **14b**: R = $COOCH_2CH_3$, R^1 = $CH{=}CHCOOCH_2CH_3$ (E + Z), n = 1, R^2 = R^3 = R^4 = H;
- Compound **14c**: R = $COOCH_2CH_3$, R^1 = $CH{=}CH_2$, n = 1, R^2 = R^3 = R^4 = H;
- Compound **14d**: R = $COOCH_2CH_3$, R^1 = CH=CHBr (E + Z), n = 1, R^2 = R^3 = R^4 = H;
- Compound **18**: R = 3'-methyl-1',2',4'-oxadiazol-5'-yl, R^1 = I, n = 0, R^2 = CH_2OH, R^3 = R^4 = OH;
- Compound **19**: R = COOH, R^1 = I, n = 0, R^2 = CH_2OH, R^3 = R^4 = OH;
- Compound **20**: R = $COOCH_3$, R^1 = I, n = 0, R^2 = $CH_2OSi(CH_3)_2$-t-Bu, R^3 = R^4 = $OSi(CH_3)_2C(CH_3)_3$
- Compound **21**: R = $CH_3CH_2CH_2CH_2OCO$, R^1 = I, n = 0, R^2 = CH_2OH, R^3 = R^4 = OH;
- Compound **23**: R = $COOCH_3$, R^1 = I, n = 0, R^2 = $CH_2OPO_3Na_2$, R^3 = R^4 = OH;
- Compound **25**: R = $COOCH_3$, R^1 = I, n = 0, R^2 = $CH_2OPO[OCH_2OCOC(CH_3)_3]_2$, R^3 = R^4 = OH;
- Compound **27**: R = $COOCH_3$, R^1 = I, n = 0, R^2 = CH_2OH, R^3 and R^4 = isopropylidene;

- Compound **29**: R = $COOCH_3$, R^1 = I, n = 0, R^2 = $CH_2OPO[OCH_2CH_2SCOC(CH_3)_3]_2$, R^3 and R^4 = isopropylidene;
- Compound **31**: R = $COOCH_3$, R^1 = I, n = 0, R^2 = $CH_2OPO[OCH_2CH_2SCOC(CH_3)_3]_2$, R^3 = R^4 = OH;

Compounds of general formula (I) may be prepared by the processes described below (in the formulae Me indicates the CH_3 group, Et the CH_3CH_2 group and Bu the $CH_3CH_2CH_2CH_2$ group).

The synthesis of iodo- and ethynyl-pyrazole nucleosides **2** and **3**, see Scheme 1, starts in both cases from the protected intermediate **1** (which was obtained as described by Manfredini *et al.* (J. Med. Chem., 1992, *35*, 917-924). The treatment with sodium methoxide in methanol affords the deprotected methyl carboxylate derivative **2**.

Scheme 1

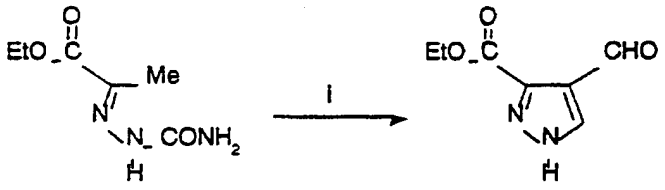


i: $Pd(PPh_3)_2Cl_2$, trimethylsilylacetylene, Et_3N, 60°; ii: MeONa/M OH.

The ethynyl derivative 3 may be prepared by coupling reaction between 1 and, for example, (trimethylsilyl)acetylene with a catalytic amount of bis (triphenylphosphine) palladium dichloride and CuI in organic solvents such as triethylamine at a temperature range of 60–80° C.

The new intermediate 4 (4–formyl–3–ethoxycarbonyl pyrazole), previously unreported, may be prepared (Scheme 2) starting from an appropriate semicarbazone such as ethylpyruvate semicarbazone, following a procedure described by Kira *et al.*, J. Heterocyclic Chem., 1970, *7*, 25–27, and modified by us, using as reagent for example $POCl_3$ in an organic solvent such as DMF.

Scheme 2



4

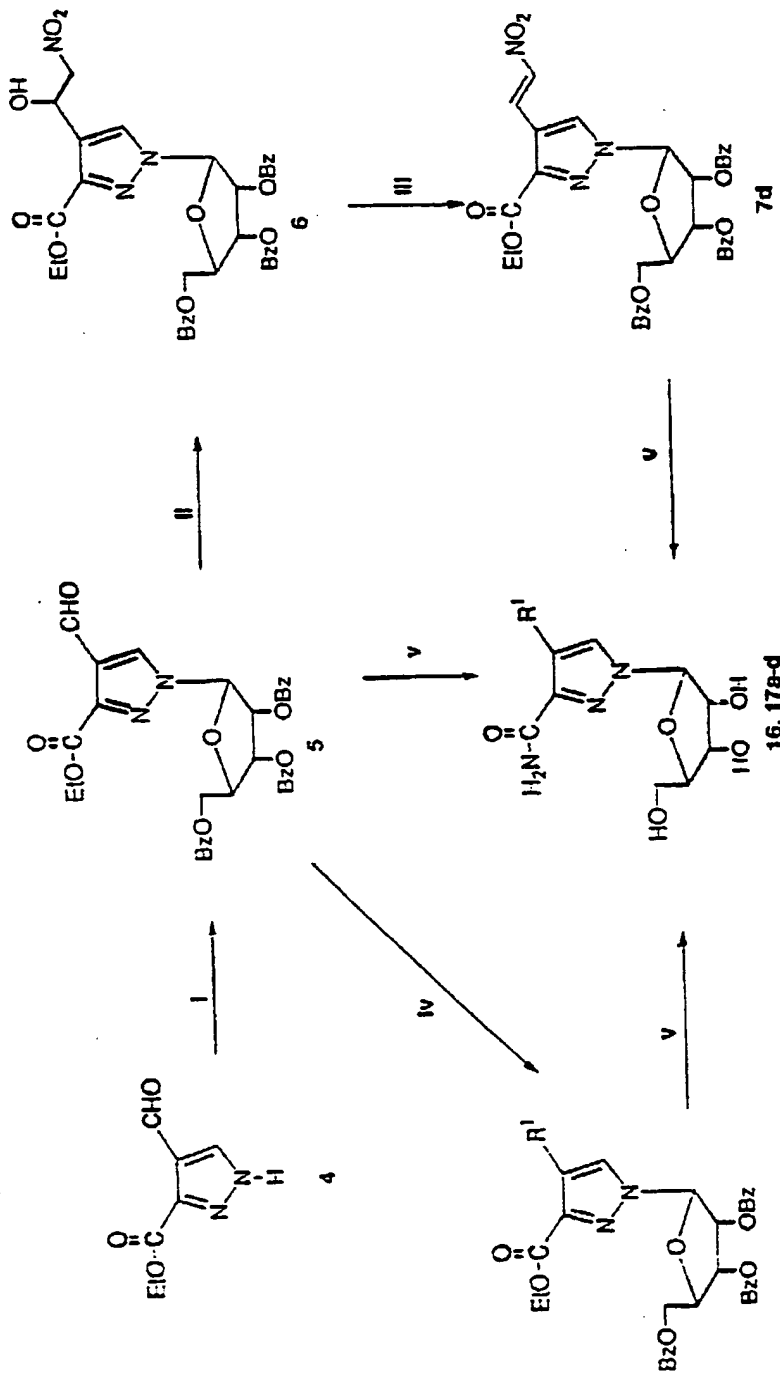
i: $POCl_3$, DMF

The glycosylation reaction (depicted in Scheme 3) may be carried out following the procedure described by Manfredini *et al.* (J. Med. Chem., 1992, *35*, 917–924). Namely by silylating the heterocycle with hexamethydsilazane and then treating with 1–*O*–acetyl–2,3,5–tri–*O*–benzoyl–β–D–ribofuranose in the presence of trimethylsilyl, chloride and trifluoromethane–sulfonate to give the protected nucleoside 5, the key intermediate for the preparation of compounds 6 and **7a–d**.

The synthesis of derivatives **7a–c** may be performed through a Wittig reaction for example of carboxyethylmethylene phosphorane (a), or the appropriate triphenylphosphonium bromide derivative (b,c), and the aldehyde 5. The isomer (E) is predominant (73:5, E:Z) in the case of 7a, whereas a 1:1 E:Z mixture is obtained in the case of 7c.

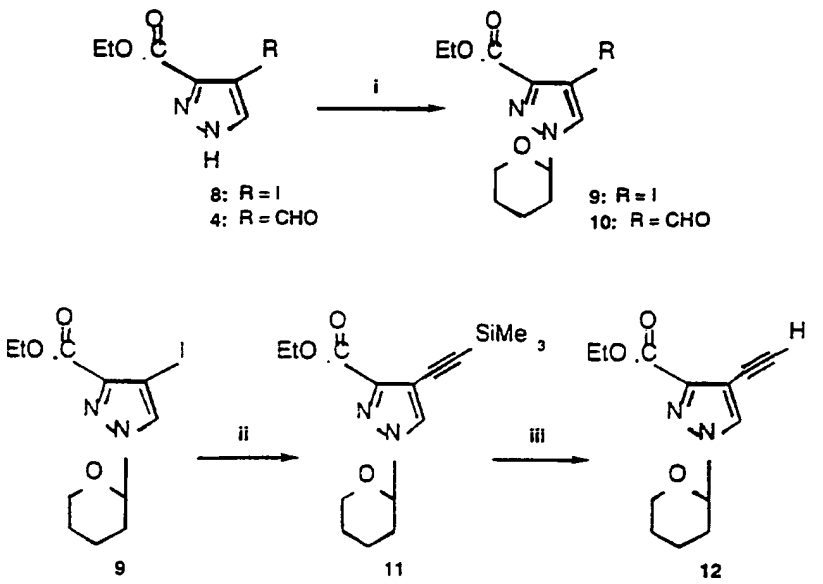
Compound 5 may be reacted for example with aluminium (III) oxide and nitromethane to give the β–nitroalcohol 6; this may be in turn converted into the nitrovinyl derivative **7d** upon dehydration, preferentially with mesylchloride.

Scheme 3


a: R^1 = –CH=CH–CO_2 Et; b: R^1 = –CH=CH_2; c: R^1 = –CH=CHBr; d: R^1 = –CH=CH–NO_2; 16: R^1 = –CHO.
I: HMDS, TMS, TfOH, MeCN, 1-acetyl-2',3',5'-tri-O-benzoyl-β-D-ribofuranose; II: $MeNO_2$, Al_2O_3;
III: $MeSO_2Cl$, TEA; IV: a: Ph_3P=CH–CO_2Et, Toluene, Δ or Ph_3P–CH_2Y^+ Br^-, BuLi, (b: Y=H, C: Y=Br);
V: NH_3/MeOH

The steps allowing to obtain compounds **9–12** are depicted in Scheme 4. The starting compounds are **4** and **8**. The 4–iodo–3–ethoxycarbonyl pyrazole (**8**) may be obtained as described by Manfredini *et al.* (J. Med. Chem., 1992, *35*, 917–924). The intermediates **9** and **10** may be obtained by the acid–catalyzed reaction of **4** and **8**, respectively, for example with 2,3–dihydro–4H–pyran (DHP) in the presence of p–toluene–sulfonic acid (TsOH). The ethynyl derivative **11** may be obtained by coupling reaction between compound **9** and, for example, (trimethylsilyl)acetylene with a catalytic amount of bis(triphenylphosphine)palladium dichloride and CuI in an organic solvent such as triethylamine at a temperature range of 60–80° C, preferentially at 60° C. Compound **11** may be in turn deprotected at the ethynyl group, for example by treatment with tetrabutylammoniumfluoride (TBAF) under standard conditions, to give the compound **12**.

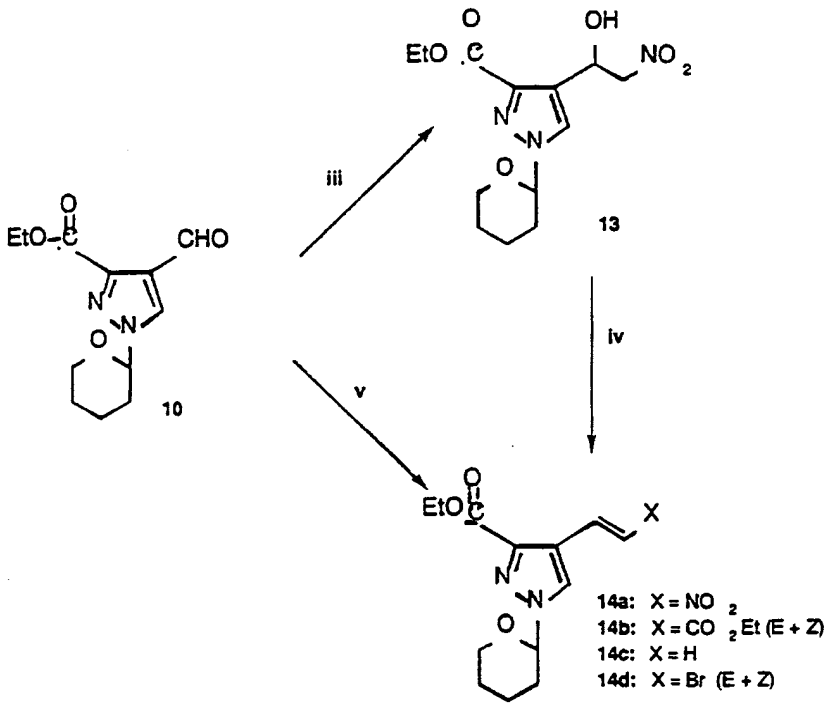
Scheme 4



i: DHP, $CHCl_3$, pTSOH; ; ii: $Pd(PPh_3)_2Cl_2$, trimethylsilylacetylene, Et_3N, 60°;
iii: TBAF/THF

As depicted in Scheme 5, the formyl derivative **10** may be treated for example with aluminium (III) oxide and nitromethane to afford the β–nitroalcohol **13** (65% yield), which may be in turn converted into the nitrovinyl derivative **14a** upon dehydration performed for example with mesylchloride.

Scheme 5

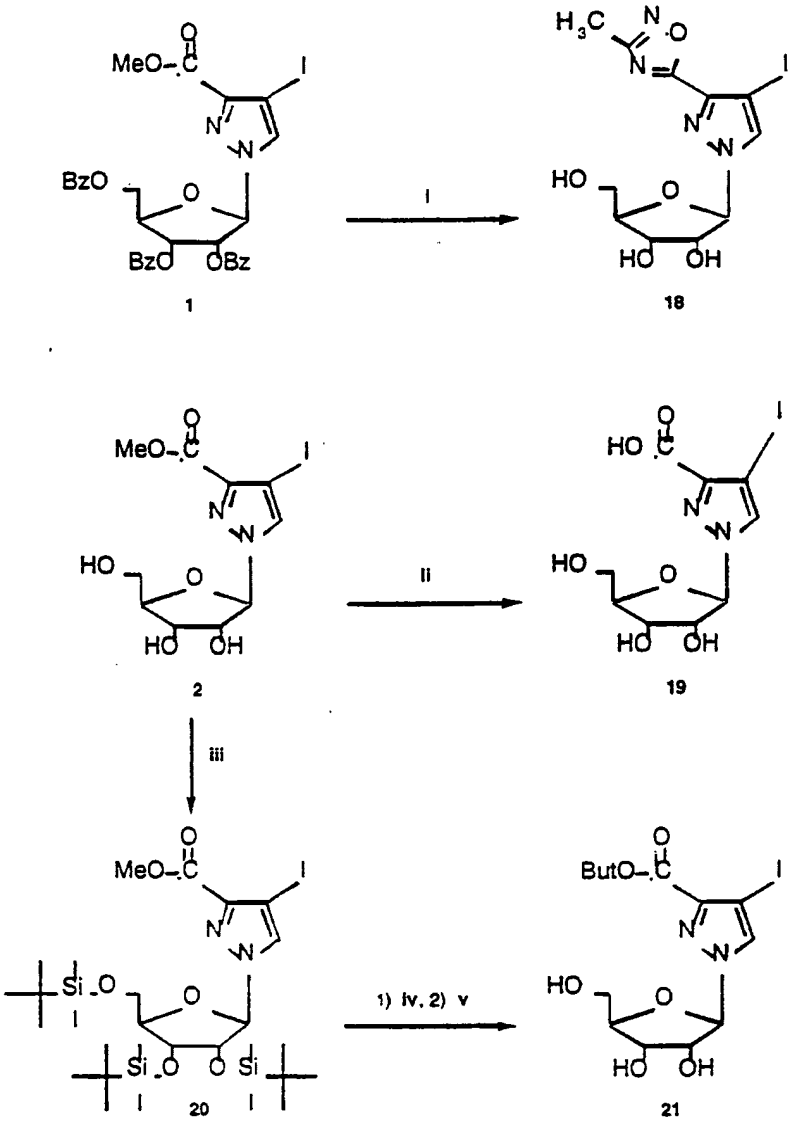


iii: $MeNO_2$, Al_2O_3; iv: $MeSO_2Cl$, TEA; v: $Ph_3P{=}CHCO_2Et$ (**b**) or $Ph_3P{-}CH_2Y^+\ Br^-$ Y=H (**c**), Y=Br (**d**), BuLi

Vinyl derivatives **14b–d** may be synthesized as described above for compounds **7a–c** by the Wittig reaction for example of carboxyethylmethylene phosphorane (**b**), or the appropriate triphenylphosphonium bromide derivative (**c, d**), with the aldehyde **10**. Again, the isomer (E) is predominant (73:5, E:Z) in the case of **14b**, whereas a 1:1 E:Z mixture is obtained in the case of **14d**.

The 3–methyl–1,2,4–oxadiazole derivative **18** may be prepared from the protected methyl ester **1** (Scheme 6) by reaction for example with acetamidoxime in the presence of a base such as sodium hydride. Under these conditions, the benzoyl protecting groups may be concomitantly removed to afford in a single step the compound **18**.

Scheme 6

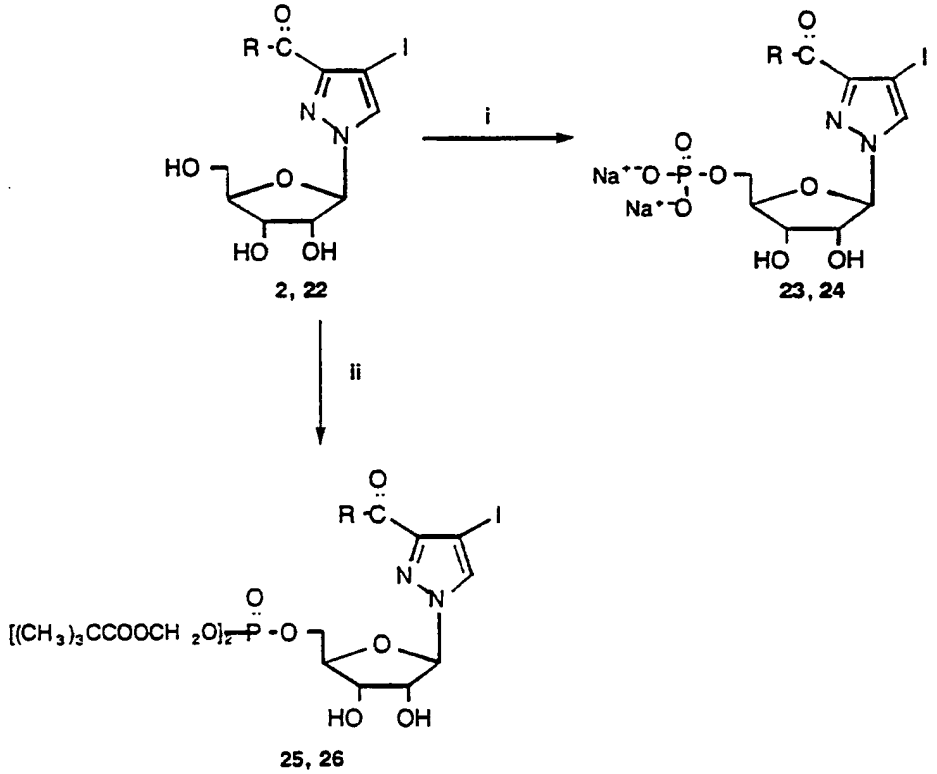


i: acetamidoxime, NaH. Δ; ii: NaOH/ H_2O; iii: TBDMSCl, DMF; iv: ButOH, NaH. Benzene; v: NH_4F, THF, AcOOH

The carboxylic acid derivative 19 (Scheme 6) may be easily obtained by hydrolysis of 2 with a base, for example sodium hydroxide, preferentially at concentrations of 0.35 M. In order to prepare the corresponding butyl ester 21, the hydroxyl groups of the sugar moiety of compound 2 may be protected in a conventional manner, for example with

terbutyldimethylsilylchloride (TBDMS). The persilylated intermediate **20** may be treated for example with an alcohol R"OH wherein R" is C_2–C_5 alkyl, preferably butanol and sodium hydride in an organic solvent such as benzene to afford the protected butyl ester. Silyl protecting groups may be removed by conventional means, conveniently with ammoniumfluoride (NH_4F) in methanol, preferentially in the presence of acetic acid, to give the expected compound **21**

Scheme 7



2, 23, 25: R = CH_3O; **22, 24, 26:** R = NH_2

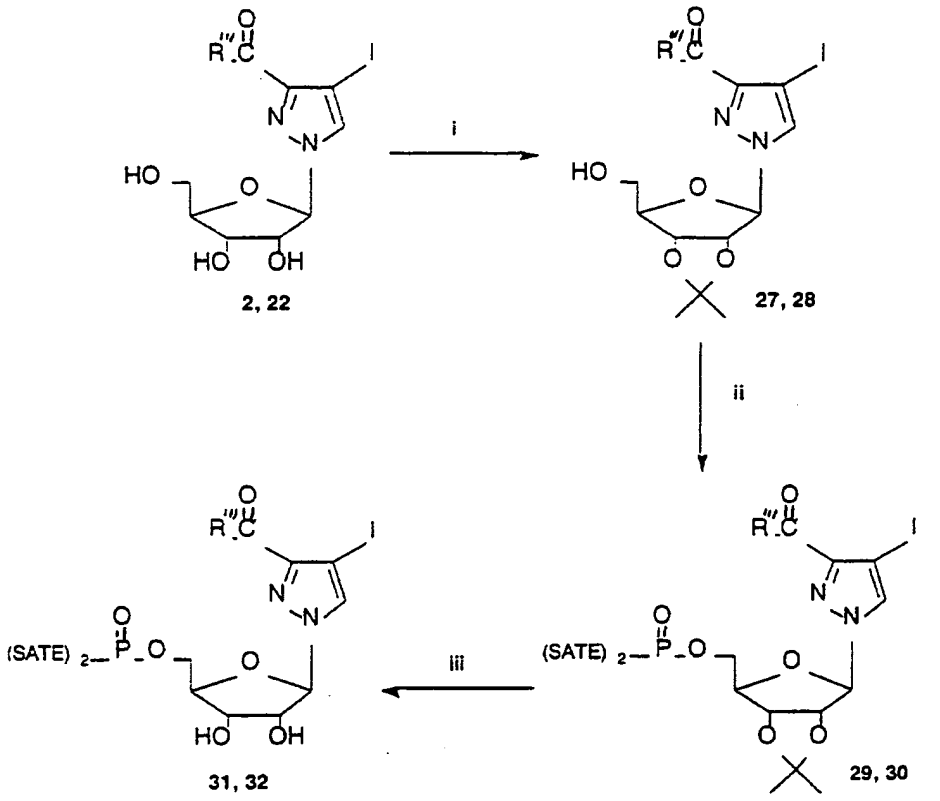
i: $POCl_3$, Py, CH_2CN, H_2O;
ii: (pivaloyloxymethyloxy)$_2$POOH, Ph_3P, $(EtO_2C)N_2$, $CH_3CON(CH_3)_2$

Regioselective introduction of monophosphate on 5' hydroxyl groups of the deprotected derivatives 2 (Scheme 7) may be effected by conventional methods, for example by means of a phosporylating agent generated in situ starting from $POCl_3$ and pyridine in the presence of polar solvents such as acetonitrile and water and subsequently salyfing the obtained product with cationic exchange resin thereby obtaining the disodium salt **23**.

The 5'-bis[(pivaloyloxy)methyl] monophosphate derivative **25** [abbreviated as bis(POM) phosphotriester] may be obtained starting from the deprotected nucleosides **2** by coupling with bis[(pivaloyloxy)methyl] hydrogen phosphate, prepared in an organic solvent such as dimethylacetamide in the presence, for example, of triphenylphosphine and diethyl azodicarboxylate preferably at 60 °C (Scheme 7).

The synthesis of the phosphotriesters 5'-bis(S-pivaloyl-2-thioethyl) monophosphate **31** [abbreviated as bis(SATE) phosphotriesters], reported in Scheme 8, may be performed starting from the protected derivatives **27**. The latter may be in turn obtained starting from nucleosides **2** starting from 2,2-dimethoxypropane in the presence of bis-(p-nitrophenyl)-phosphate in an organic solvent such as acetone, preferentially anhydrous, to give the corresponding 2',3'-O-isopropylidene derivatives. The free 5'-hydroxyl groups may be reacted with the bis(S-pivaloyl-2-thioethyl) N,N-diisopropyl-phosporamidite in an organic solvent such as THF, in the presence, for example, of 1H-tetrazole at room temperature. The phosphite group may be then oxidized for example with t-butylhydroperoxyde to afford the protected bis(SATE) phosphotriesters **29**. The removal of the protecting group may be carried out by conventional means such as acid hydrolysis, conveniently by reaction with for example TsOH in a polar organic solvent such as methanol, to give the espected compounds **31**.

Scheme 8



2, 27, 29: R''' = CH_3O; 22, 28, 30 R = NH_2; SATE = $[(CH_3)_3CCOSCH_2CH_2O]_2$

i: $(CH_3)_2CH(OCH_3)_2$, BNPP, acetone;
ii: $(SATE)_2$-P-N-(i-Pr)$_2$, 1*H*-Tetrazole, THF, t-BuOOH;
iii: TsOH, MeOH

MATERIALS AND METHODS

Compounds. The compounds used in this study were solubilized in DMSO at an initial concentration of 100 mg/mL and then serially diluted in RPMI 1640.

Cells. Cell lines were purchased from the American Type Culture Collection (ATCC). Leukemia- and lymphoma-derived cells were grown in RPMI-1640 medium supplemented with 10% FCS, 100 units/mL penicillin and 100 μg/mL streptomycin. Cell cultures were incubated at 37° C in a humidified, 5% CO_2 atmosphere. The cell lines used were the following: L1210, mouse leukemia; Wil2-NS, human splenic B-lymphoblastoid cells; CCRF-SB, human acute B-lymphoblastic leukemia; Raji, human Burkitt lymphoma; CCRF-CEM and MOLT-4, human acute T-lymphoblastic leukemia; C8166 and MT-4, human CD4+ T-cells containing an integrated HTLV-1 genome.

Solid tumor-derived cells were grown in their specific media supplemented with 10% FCS and antibiotics. The cell lines used were the following: CHO-K1, chinese hamster ovary; HT-29, human colon adenocarcinoma; HeLa, human cervix carcinoma; ACHN, human renal adenocarcinoma; 5637, human bladder carcinoma; IMR-32, human neuroblastoma. Cell cultures were incubated at 37° C in a humidified, 5% CO_2 atmosphere.

The absence of mycoplasma contamination from both suspension and monolayer cultures was checked periodically by the Hoechst staining method.

Viruses. Human immunodeficiency virus type-1 (HIV-1. HTLV/III_B strain) was obtained from supernatants of persistently infected H9/III_B cells. The HIV-1 stock solution had titres of $4.5x10^6$ cell culture infectious dose fifty (CCID50)/mL. The other viruses used in this study were purchased from the ATCC. Stock solutions of herpes simplex types 1 and 2 (HSV-1. HSV-2), vaccinia. Vesicular Stomatitis Virus (VSV), Coxsackie B2, were prepared in Vero cells and had titres of $5.0x10^7$, $1.0x10^8$, $2.0x10^7$, $4.0x10^8$ and $1.0x10^8$ plaque forming units (PFU) / mL. respectively.

Antiproliferative assays. Exponentially growing leukemia and lymphoma cells were resuspended at a density of $1x10^5$ cells/mL in growth medium containing serial dilutions of the drugs, alone or in combination. Cell viability was determined after 96 hrs at 37 °C by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method. as previously described by Denizot F. and Lang R. (J. Immunol. Methods 89, 271-277. 1986) and by Pauwels *et al.* (J. Virol. Methods 20. 309-321. 1988).

Activity against solid tumor-derived cells was evaluated in exponentially growing cultures seeded at $5x10^4$ cells/mL and allowed to adhere for 16 hrs to culture plates before addition of the drugs. Cell viability was determined by the MTT method four days later. Cell growth at each drug concentration was expressed as percentage of untreated controls and the concentration resulting in 50% growth inhibition (IC_{50}) was determined by linear regression analysis. The effect of drug combinations was evaluated by isobolograms. FIC indexes were calculated for each concentration ratio according to Suhnel J. (Antiviral Res. 13, 23-40, 1990).

Cytotoxicity assays. Peripheral blood lymphocytes (PBL) from HIV-negative donors were obtained by separation on Fycoll-Hypaque gradients. After extensive washings, cells were resuspended ($1x10^6$ cells/mL) in RPMI-1640 with 10% FCS and incubated overnight.

For cytotoxicity evaluations in proliferating PBL, non-adherent cells were resuspended at $1x10^6$ cells/mL in growth medium, stimulated with PHA (2.5 μg/mL) for 24 hrs before dilution to $1x10^5$ cells/mL in medium containing PHA (2.5 μg/mL), IL-2 (50 U/ml) and various concentrations of the test compounds. Viable cell numbers were determined six days later. Under these conditions, untreated PBL were able to undergo exponential growth up to four cell cycles, as determined by viable cell counts.

For cytotoxicity evaluations in resting PBL, non-adherent cells were resuspended at high density ($1x10^6$ cells/mL) and treated for as long as 3 days with the test compounds. Then, the cells were extensively washed to remove the inhibitors and were stimulated with PHA for 24 hrs before being diluted to $1x10^5$ cells/mL in medium containing PHA and IL-2. Cell viability was determined after incubation at 37°C for six days.

Myeloid precursors of granulocyte-monocytes (CFU-GM) were obtained from the bone marrow of healthy donors by separation on Fycoll-Hypaque gradients. After extensive washings, cells ($2x10^5$/mL) were resuspended in Iscove medium containing 0.3% agar and growth factors from the supernatant of 5637 cells. After ten days at 37° C, colonies (composed of about 40 cells) of CFU-GM were scored under the light microscope.

Anti-HIV assays. Activity of compounds against the HIV multiplication in acutely infected cells was based on inhibition of the virus-induced cytopathogenicity in MT-4 cells. Briefly, 50 μL of culture medium additioned of 10% FCS containing $1x10^5$ MT-4 cells (infected de novo with HIV-1 at a multiplicity of infection of 0.01) were added to each well of flat bottomed microtitre trays containing 50 μL of medium with or without serial dilutions of the test compounds, alone or in combinaton. After 4 days incubation at 37° C, the number of

viable MT-4 cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method (22). Cytotoxicity of compounds was evaluated in parallel with their antiviral activity and was based on the viability of mock-infected cells, as monitored by the MTT method.

Antiviral assays. The antiviral activity against HSV-1 and -2 was evaluated by either the plaque reduction assay in Vero cells or the yield reduction assay in Hela cells. The activity against vaccinia, VSV and Coxsackie B_2 viruses was evaluated by the MTT method in Vero cells infected at a multiplicity of infection of 0.01.

Kaposi's model in mice. Kaposi's sarcoma secreted products (KS_{sup}) were obtained from cell-free supernatants of cell cultures from Kaposi's lesions as previously described by Albini A., et al. (Int. J. Oncol. 1, 723-730, 1992). The standard amount of KS_{sup} inoculated per sample corresponded to the culture supernatant of $4x10^5$ cells. Liquid Matrigel solutions containing KS_{sup}, heparin (22 U/ml) and various concentrations of the test compounds were injected subcutaneously into the flanks of C57bl/6 male mice (final volume 600 µl) where they formed a polymerized support. Five days after injection, the animals were sacrificed and the gels were recovered and weighted. The haemoglobin content of these gels was measured using a Drabkin reagent Kit 525 (Sigma) and its concentration, determined using a standard curve. was normalized to 100 mg of the recovered gel.

BIOLOGICAL ACTIVITY

Antiproliferative activity of compounds of general formula (I), in vitro. The antiproliferative effect of selected compounds of the present invention was evaluated against a panel of human leukemia, lymphoma and solid tumor cell lines, together with that of ribavirin, selenazofurin and doxorubicin, used as reference drugs (Table 1).

Table 1. Comparative antiproliferative activity of compounds of formula (I) and reference antitumor drugs.

Cell line	$^{a}IC_{50}$[μM]				
	2	4 / 8	Ribavirin	Selenazofurin	Doxorubicin
Leukemia/ Lymphoma					
L1210	2.4 (± 0.3)	>300	16 (± 5)	0.9 (± 0.6)	0.9 (± 0.5)
Wil2-NS	2.1 (± 0.1)	>300	>100	1.5 (± 0.4)	0.03 (±0.02)
CCRF-SB	1.7 (± 0.2)	>300	>100	1.2 (± 0.4)	0.05 (± 0.04)
Raji	0.2 (± 0.1)	>300	56 (±6)	0.8 (± 0.2)	0.1 (± 0.05)
CCRF-CEM	1.5 (± 0.3)	>300	19(± 8)	2.9 (± 0.3)	0.08 (± 0.01)
MOLT-4	0.8 (± 0.2)	>300	5.7 (± 0.9)	0.7 (± 0.2)	0.05 (± 0.03)
C8166	0.2 (± 0.2)	>300	>100	0.8 (± 0.3)	0.05 (± 0.07)
MT-4	1.6 (± 0.2)	>300	23 (±6)	1.2 (± 0.4)	0.05 (± 0.03)
Carcinoma					
CHO-K1	8.6 (± 2.2)	>300	50 (± 8)	8.5 (± 1.9)	0.2 (± 0.1)
HT-29	2.9 (± 1.1)	>300	ND	4.3 (± 0.8)	0.9 (± 0.5)
HeLa	5.0 (± 1.7)	>300	≥100	3.2 (± 1.3)	ND
ACHN	3.6 (± 0.9)	>300	>100	9.5 (± 2.1)	0.1 (± 0.07)
5637	5.4 (± 1.5)	>300	46 (±5)	7.4 (± 1.2)	0.03 (± 0.05)
Neuroblastoma					
IMR-32	3.3 (± 0.3)	>300	3.3 (± 0.3)	4.7 (± 0.3)	0.03 (± 0.02)

[a]Inhibitory concentration fifty: compound concentration (± SD) required to reduce cell growth by 50% under conditions allowing untreated cell controls to undergo at least three consecutive rounds of multiplication.

L1210, mouse leukemia; Wil2-NS, human splenic B-lymphoblastoid cells; CCRF-SB, human acute B-lymphoblastic leukemia; Raji, human Burkitt lymphoma; CCRF-CEM and MOLT-4, human acute T-lymphoblastic leukemia. C8166 and MT-4, human $CD4^+$ T-cells containing an integrated HTLV-1 genome; CHO-K1, chinese hamster ovary; HT-29, human colon adenocarcinoma; HeLa, human cervix carcinoma; ACHN, human renal adenocarcinoma; 5637, human bladder carcinoma; IMR-32, human neuroblastoma.

Compound 2 showed potent inhibition of leukemia and lymphoma cell proliferation (IC_{50} in the range 0.2-2.4 μM) and turned out only 2-4 times less potent against carcinoma and neuroblastoma cell lines (IC_{50} in the range 2.9-8.6 μM). Overall, no significant differences could be seen in both potency and spectrum of antiproliferative activity between 2 and selenazofurin. Doxorubicin was the most potent compound (IC_{50} in the range 0.03-0.9 μM), ribavirin the weaker (IC_{50} in the range 5.7-27 μM). On the other hand, compounds 4 and 8 resulted totally inactive, allowing to conclude that the withdrawal of ribose from compounds of formula (I) abates tha antiproliferative activity (see compounds 4 and 8). However, when ribose is substituted for by a tetrahydropyranyl group (i.e. compounds of formula (I) wherein n = 1), the antiproliferative activity is restored (see Table 7). Among these derivatives, the more active compounds were those carrying the following groups: $CH=CHNO_2$, CH=CHBr or CHO at position 4 in the pyrazole ring.

Table 2. Antiproliferative activity of compounds of formula (I) wherein n = 1.

Cell line	[a]IC_{50}[μM]								
	9	10	11	12	14a	14b(E)	14b(Z)	14c	14d
Leukemia/ Lymphoma									
L1210	>300	38	>300	>300	23	>300	>300	>300	>300
Wil2-NS	>300	21	>300	>300	14	>300	>300	>300	102
CCRF-SB	>300	32	>300	>300	16	230	180	>300	89
Raji	>300	32	>300	>300	24	>300	>300	>300	91
C8166	>300	22	>300	>300	24	>300	>300	>300	100
MT-4	>300	30	>300	>300	25	>300	>300	>300	28
Carcinoma									
CHO-K1	>300	43	>300	>300	17	230	180	>300	91
HT-29	>300	64	>300	>300	26	>300	>300	>300	102
HeLa	>300	100	>300	>300	29	>300	>300	>300	255
ACHN	>300	51	>300	>300	17	>300	>300	>300	155

Inhibitory concentration fifty: compound concentration (± SD) required to reduce cell growth by 50% under conditions allowing untreated controls to undergo at least three consecutive multiplication rounds. Values are the mean of three separate experiments. Variability among triplicate samples was less than 12%.

L1210, mouse leukemia; Wil2-NS, human splenic B-lymphoblastoid cells; CCRF-SB, human acute B-lymphoblastic leukemia; Raji, human Burkitt lymphoma; C8166 and MT-4, human $CD4^+$ T-cells containing an integrated HTLV-1 genome; CHO-K1, chinese hamster ovary; HT-29, human colon adenocarcinoma; HeLa, human cervix carcinoma; ACHN, human renal adenocarcinoma.

Cytotoxicity of compounds of formula (I) in normal human cells, in vitro. In order to get more insights into the cytotoxicity of **2**, the drug was tested in vitro against peripheral blood lymphocytes (PBL), either before or after PHA stimulation, and against bone marrow granulocyte/monocyte (CFU-GM) precursors from healthy donors. Selenazofurin and various anticancer agents of current clinical use were run as reference drugs (Table 2).

Table 3. Comparative cytotoxicity of compound **2** and reference anticancer drugs in human cells.

Cells	[a]CC_{50} [μM]					
	2	Selenazofurin	MTX	Cis-Pt	Doxorubicin	Taxol
[b]PBL_{PHA}	2.6 (± 1.0)	1.0 (± 0.5)	0.05 (± 0.05)	10 (± 4)	0.04 (± 0.06)	0.005 (± 0.004)
[c]$PBL_{resting}$	500 (± 50)	100 (± 35)	0.1 (± 0.08)	16 (± 4)	0.09 (± 0.05)	2.4 (± 1.2)
[d]CFU-GM	*12 (± 2)	*0.1 (± 0.05)	ND	ND	0.03 (± 0.02)	ND
[e]Leukemia	1.5	1.2	0.01	2.6	0.1	0.007

[a]Cytotoxic concentration fifty: compound concentration (± SD) required to reduce cell multiplication (PBL) or colony formation (CFU-GM) by 50%.

[b]PBL were stimulated with PHA and then resuspended in IL_2-containing medium in the presence of the drugs.

[c]PBL were treated with test drugs for 3 days and then were stimulated with PHA and resuspended in drug-free medium.

[d]CFU-GM, colony forming units of bone marrow hematopoietic progenitors of granulocytes, macrophages.

[e]Data are the mean IC_{50} values obtained in leukemia cell lines.

Compound **2** proved cytotoxic (CC_{50} = 2.6 µM) for PHA-stimulated PBL at concentrations similar to those active against leukemic cells (mean IC_{50} = 1.5 µM). On the contrary, following a 3 day treatment with of compound **2** at 500 μM, resting PBL maintained unaltered their capability to proliferate upon removal of the drug and stimulation with PHA. Under the

same conditions, only selenazofurin and the antimitotic drug taxol showed a selective inhibition of proliferating PBL, whereas MTX, cis-platinum and doxorubicin proved equally cytotoxic to proliferating and resting lymphocytes. Moreover, when the CC_{50} of each compound for myeloid precursor cells were evaluated, **2** was less cytotoxic than selenazofurin and doxorubicin. Therefore, compound **2** proved comparable to selenazofurin and well-inferior to doxorubicin in terms of potency and spectrum of antiproliferative activity, and significantly less cytotoxic against PBL and bone marrow haematopoietic precursors.

Effect of 2 in combination with various antitumor drugs. In view of the structural similarities shared by **2**, tiazofurin and ribavirin, the antiproliferative effects of these compounds in combination with each other were evaluated in CEM cells.

Table 4. Antiproliferative effect of compound 2 in combination with other anticancer drugs.

Drug	[μM]	Viability (% of untreated controls)	
		[a]Observed	[b]Predicted
2	0.75	82	
araA	25	25	
2 + araA	0.75 + 25	21	20
6MP	0.5	64	
2 + 6MP	0.75 + 0.5	[d]18	52
6TG	1.25	53	
2 + 6TG	0.75 + 1.25	48	43
[c]2 + 6TG	0.75 + 1.25	[d]23	43
5FU	4.5	77	
2 + 5FU	0.75 + 4.5	57	63
[c]2 + 5FU	0.75 + 4.5	[d]36	63
MTX	0.01	31	
2 + MTX	0.75 + 0.01	21	25
Doxorubicin	0.025	37	
2 + Doxorubicin	0.75 + 0.025	22	30
Taxol	0.008	31	
2 + Taxol	0.75+ 0.008	20	25

[a]Values are the mean of three separate experiments carried out in CEM cells. Variability among triplicate samples was less than 12%.

[b]Value predicted for additive effect.

[c]Cells were pretreated with compound 2 for 12 hrs.

[d]Significant synergism (the observed value is less than 70% of the predicted value).

Within a wide concentration range, compound 2 proved synergistic with ribavirin and additive with tiazofurin or selenazofurin: on the other hand, tiazofurin confirmed synergistic in combination with ribavirin and additive with selenazofurin (data not shown).

We then investigated the effects of simultaneous and sequential treatments of CEM cells with 2 and each of several antitumor agents with different modes of action (Table 4). Following simultaneous treatment, combinations of 2 with 6MP resulted in a synergistic effect. By contrast, paired combinations of compound 2 with each of the other antimetabolites araA, 6TG, 5FU and MTX, the alkylating agent cisplatinum, the intercalating compound doxorubicin or the antimitotic drug taxol gave additive antiproliferative effects. Interestingly, when the cultures were pretreated for 12 hrs with compound 2 before the addition of the second antitumor agent, the pyrazole derivative proved synergistic also in combination with 6TG and 5FU.

Susceptibility to other antitumor drugs of cells resistant to compound 2. Cells resistant to compound 2 (ACHN/R1) were established by cultivation of sensitive ACHN cells in medium containing stepwise increasing concentrations of the drug. A stable clone of ACHN/R1 cells showing an IC_{50} 30 fold higher than that of the parental cell line was obtained after 4 months from the first exposure to compound 2 at 3 μM . Before evaluating the sensitivity of ACHN/R1 cells to the other antitumor agents, cells resistant to 2 were allowed to grow for three cell cycles in drug-free medium. As shown in Table 5, cells resistant to 2 proved fully susceptible to inhibition by all anticancer drugs tested with two exceptions: 6TG and 5FU, towards which ACHN/R1 were 7-fold and 2-3 fold more resistant than the parental cell line, respectively. Viceversa, 2 proved efficacious in inhibiting both KB^{VCR} and KB^{VP16} cells expressing the MDR phenotype (results not shown).

Potentiation of the anti-HIV-1 activity of antiretroviral drugs by compound 2. When compound 2 was tested against HIV-1, it showed no antiviral activity per se. However, when tested in combination with 2',3'-dideoxyinosine (ddI), 2',3'-dideoxyadenosine (ddA), 9-[2-

(phosphonomethoxy)ethyl]adenine (PMEA), 9-[2-(phosphonomethoxy)propyl]guanine (PMPG), 9-[2-(phosphonomethoxy)propyl]adenine (PMPA), (Table 6), compound 2 was found to enhance the anti-HIV-1 activity of all these drugs. Therefore, the present invention refers to a pharmaceutical composition comprising a compound of the present invention, preferentially compound 2, in combination with antiretroviral drugs such as dideoxynucleosides or acyclic phosphonate nucleosides for the AIDS therapy. In conclusion, combinations of compound 2 with ddI, PMEA, PMPG or PMPA appear to be promising in particular for the therapy of AIDS patients with or without AIDS-associated neoplasias.

Table 5. Susceptibility to other anticancer drugs of cells resistant to compound 2.

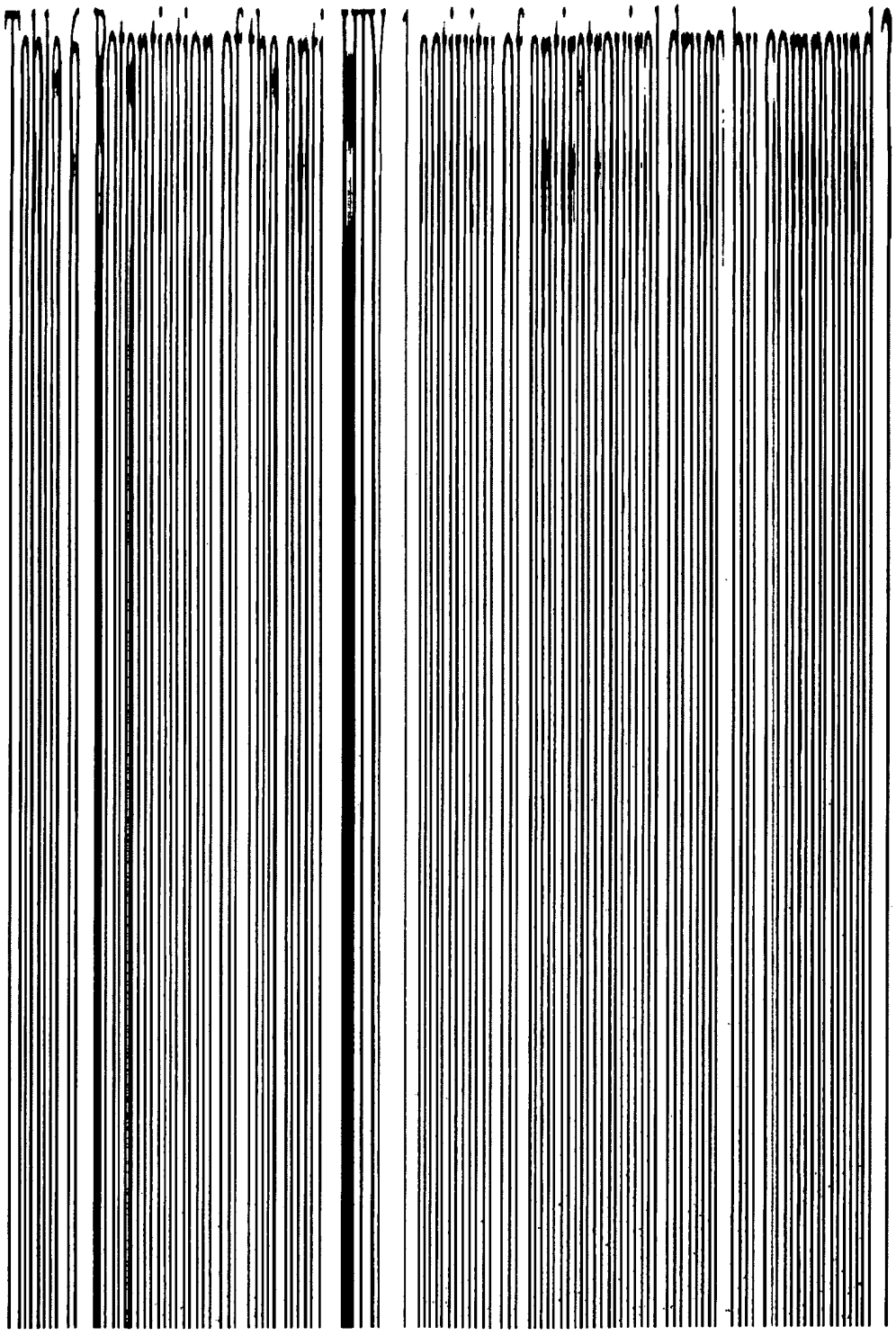
Compound	[a]IC_{50} [μM]	
	ACHN	[b]ACHN/R1
2	3.6 (± 0.9)	80 (± 7)
Ribavirin	130 (± 10)	140 (± 12)
Selenazofurin	10 (± 2)	9 (± 2)
araA	116 (± 10)	92 (± 8.3)
6MP	6.4 (± 1.2)	6.8 (± 1.6)
6TG	7.1 (± 1.0)	44 (± 5.2)
5FU	8.3 (± 1.7)	18 (± 2.1)
MTX	0.08 (± 0.05)	0.07 (± 0.06)
Cis-Pt	5.0 (± 0.8)	3.2 (± 0.5)
Doxorubicin	0.13 (± 0.05)	0.12 (± 0.08)
Taxol	0.16 (± 0.09)	0.1 (± 0.04)

[a]Inhibitory concentration fifty: compound concentration (± SD) required to reduce cell multiplication by 50% under conditions allowing untreated controls to undergo at least three consecutive multiplication rounds.

[b]Cell subclone resistant to compound 2 established by cultivation of ACHN cells in medium containing stepwise increasing concentrations of this drug.

Compounds of formula (I), namely 2, 4 and 8 were evaluated for their antiviral activity against HSV-1, HSV-2, Vaccinia, Vesicular stomatitis virus and Coxsackie B2, but none of them resulted active at concentrations as high as 300 μM (data not shown). However, when tested in

combination with acyclovir (ACG), 9-[2-(phosphonomethoxy)ethyl]guanine (PMEG) and adenine arabinoside (araA), compound 2 was found to enhance the anti-HSV-1 activity of these drugs (Table 7).



Efficacy of compound 2 on Kaposi's-like lesions induced in mice. The fact that 2 enhances the anti-HIV-1 activity of ddI, ddA, PMPG and PMPA (13) makes the pyrazole nucleoside a promising candidate for the treatment of AIDS-related neoplasias. To obtain further information on its antitumor efficacy in vivo, compound 2 was tested in a murine model of Kaposi's sarcoma, in which lesions carrying all the phenotypic hallmarks of KS are readily induced by cell-free supernatants of KS cell cultures (KS_{sup}) (25). In this model, co-injection of 4, 2 and 1 mg/kg of compound 2 with the KS_{sup}/heparin/Matrigel mixture resulted in over 80% inhibition of the angiogenic response, as measured by the haemoglobin content of Matrigel implants (Table 7). Surprisingly, doxorubicin, used as reference drug, did not affect the KS_{sup}-induced angiogenic process at 1.5 mg/kg.

Table 7. Potentiation of the anti-HSV-1 activity of antiherpes drugs by compound 2.

Treatment	[a]PFU/mL	
	2	antiretroviral drugs
2 (5.0 μM)	$4.5x10^5$	
ACG (5.0 μM)		$4.2x10^4$
PMEG (2.0 μM)		$8.6x10^4$
araA (25 μM)		$1.5x10^5$
2 (5.0 μM)+ACG (5.0 μM)		$5.1x10^3$
2 (5.0 μM)+PMEG (2.0 μM)		$4.1x10^3$
2 (5.0 μM)+araA (25 μM)		$2.9x10^4$

[a]Plaque forming units/mL, i.e. virus titre obtained in the presence of compounds, alone or in combination, in HeLa cell monolayers infected with HSV-1 at a multiplicity of infection of 0.01. Values are the mean for three separate experiments. Variability among duplicate samples was less than 10% The titre of HSV-1 in unfected, untreated controls was $5.5x10^5$ PFU/mL.

Table 7.Effect of compound 2 on the angiogenesis induced in mice by Kaposi's cell supernatants.

Compd	Median Hb content of matrigel implants	Positive gels
Matrigel alone	0.194	0/3
Heparin	0.129	0/4
[a]KS_{sup} + heparin	[b]0.753	6/8
KS_{sup} + hep. + 2 (1.0 mg/Kg)	0.509	[c]3/5
KS_{sup} + hep. + 2 (2.0 mg/Kg)	0.200	2/5
KS_{sup} + hep. + 2 (4.0 mg/Kg)	0.290	2/10
KS_{sup} + hep. + Doxo(1.5 mg/Kg)	0.725	5/5

[a]KS_{sup}: cell-free supernatant from Kaposi's cell cultures.

[b]The range of haemoglobin content in control Matrigel implants was 0.681 - 2.033.

[c] Values below 0.400 were considered negative.

SYNTHETIC EXAMPLES

Preparation of the compounds of the invention might become more understandable on the basis of the Examples reported below.

Reaction steps and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated F254 Merck plates with detection under 254 nm UV lamp and/or by spraying with 10% H_2SO_4/MeOH or 5% $KMnO_4/H_2O$ and heating. Column chromatographies were performed with Merck 60-200 mesh silica gel. Ionic exchange resins used were: Sephadex-DEAE, 25-A NH_4^+ form and Dowex 50Wx2-200. Na^+ form. Nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) spectra were determined for solution in $CDCl_3$-DMSO-*d6* on a Bruker AC-200 spectrometer and peak positions are given in parts

per million (d) downfield from tetramethylsilane as internal standard. Melting points were obtained in open capillary tubes and are uncorrected. All drying operations were performed over anhydrous magnesium sulphate. Ultraviolet spectra were recorded on a Jasco 510 spectrometer. Analytical HPLC was performed on a Waters 600E instrument on Rainin C_{18} (Dynamax 12m) and Knauer silica gel (Eurospher 100, 5m) columns. Room temperature was 22–25°C. Microanalyses, unless indicated, were in agreement with the calculated values within ± 0.4 %

Example 1

Preparation of methyl 4-iodo-1-b-D-ribofuranosyl-pyrazole-3-carboxylate (2). A solution of sodium methoxide (sodium, 0.061 g, 2.6 mmol, in dry methanol, 20 mL) was added at 0°C to a solution of compound **1** (0.5 g, 0.76 mmol) in dry methanol (20 mL). Compound **1** was previously obtained as reported by Manfredini *et al.* (J. Med. Chem., 1992, *35*, 917–924).After 1h TLC (CH_2Cl_2/MeOH, 9/1) indicated complete reaction. The mixture was then neutralized with Dowex (50W H^+), filtered and the pad was washed with methanol (3 x 50 mL). After evaporation, the crude oil was purified by column chromatography (CH_2Cl_2/MeOH, 9/1).

2: syrup: yield 75%; UV (MeOH) nm l_{max} 205 (e 17200), 254 (e 29300); $l_{shoulder}$ 219 (e 7000): l_{min} 241 (e 2500): ^1H NMR, (DMSO-*d6*) d 3.50 (m, 2H, H5',5''); 3.80 (s, 3H. OMe); 3.91 (m. 1H, H4'); 4.11 (m. 1H, H3'); 4.27 (m, 1H, H2'); 5.00 (t, 1H, J = 5.5 Hz, OH); 5.20 (d, 1H, J = 5.4 Hz, OH); 5.60 (d, 1H, J = 5.8 Hz, OH); 5.68 (d, 1H, J = 4.4 Hz, H1'); 8.36 (s. 1H. H5).

Example 2

Preparation of methyl 1-(2,3,5-tri-O-benzoyl-b-D-ribofuranosyl)-4-[2-(trimethylsilyl)-ethynyl]pyrazole-3-carboxylate (3). Freshly distilled dry Et_3N (15 mL) was vigorously purged with argon for 15 min, and the iodo-derivative **1** (0.3 g, 0.45 mmol) was added followed by (trimethylsilyl)acetylene (0.15 mL, 1 mmol), $(Ph_3P)_2PdCl_2$ (0.007 g, 0.016 mmol), and CuI (0.0035 g, 0.018 mmol). This suspension was first stirred at room temperature for 1 h and then at 80° C for 2 h under positive argon pressure (TLC. EtOAc/ Hexane. 3:7). After 5 h TLC indicated complete reaction, the solvent was evaporated. the brown residue was dissolved in dichloromethane (100 mL), washed with 2% disodium EDTA H_2O (2 x 50 mL) and H_2O (2 x 50 mL), dried and concentrated to dryness. The

resulting brown oil was purified by chromatography (EtOAc/ Hexane, 3:7 in presence of Et_3N). Appropriate fractions were evaporated to give 3 as a yellow solid.

3: mp 68°C; yield 91 %; ^{1}H NMR ($CDCl_3$) d 0.29 (s, 9H, $SiMe_3$); 3.94 (s, 3H, OMe); 4.58 (dd, 1H, J = 12 e 5 Hz, H5'); 4.82 (m, 2H, H4', H5"); 6.00 (dd, 1H, J = 10 e 3 Hz, H3'); 6.32 (dd, 1H, J = 5 e 3 Hz, H2'); 7.65 e 8.05 (m x 2, 17H, Ar, 1H', H5).

Example 3

Preparation of 3-carboxyethylpyrazole-4-carboxaldehyde (intermediate 4). Ethylpyruvate semicarbazone (4.3 g, 25 mmol) was added, under stirring, to a mixture of phosphorus oxychloride (15 ml, 55 mmol) and dimethylformamide (13 mL, 110 mmol) previously prepared below 5°C. Starting ethylpyruvate semicarbazone (mp 216°C) was prepared by the standard procedure from semicarbazide and ethylpyruvate. The reaction mixture was heated at 60°C and 4 h later was cooled and poured into crushed ice (30 g). The mixture was then neutralized with sodium hydroxide (20 g/80 mL H_2O), heated at 60°C for 5 min, cooled and acidified to pH 6 with 1N HCl. The precipitated solid was filtered off and recrystallized from ethanol.

4: mp 167-168°C; yield 83%; ^{1}H NMR. $CDCl_3$: 1.32 (t, 3H, J=7 Hz, Ethyl); 4.34 (q, 2H, Ethyl); 8.49 (s, 1H, H5); 10.20 (s, 1H, CHO); 11.20 (sbr, 1H, H1).

Example 4

Preparation of ethyl 1-(b-D-2',3',5'-tri-O-benzoyl-ribofuranosyl)-4-formyl-pyrazole-3-carboxylate (5). Hexamethyldisilazane (1.15 mL, 5.5 mmol) and a catalytic amount of ammonium sulphate were added, under positive Argon pressure, to a mixture of 4 (0.84 g, 5 mmol) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-b-D-ribofuranose (3.8 g, 7.5 mmol) in 50 mL of freshly dry distilled acetonitrile,. The mixture was heated at reflux condition, with the exclusion of moisture, until complete dissolution and then trimethylsilyl chloride (0.75 mL, 6 mmol) and trifluoromethane sulfonic acid (1.05 mL, 12 mmol) were added. Reaction course was monitored with TLC (EtOAc:Hexane). After 3 h the reaction mixture was cooled to ambient temperature, diluted with dichloromethane (50 mL) and extracted with a satured $NaHCO_3/H_2O$ solution. The aqueous phase was then extracted with dichloromethane (2 x 50 mL) and the organic layer was washed with a satured $NaCl/H_2O$ solution, dried and evaporated. The residue was purified by column chromatography (EtOAc/Hexane, 3/7).

5: Syrup; yield 63%; ^{1}H NMR ($CDCl_3$) d 1.32 (t, 3H, J = 7 Hz. Ethyl); 4.34 (q, 2H, Ethyl); 4.65 (dd. 1H, J = 12 and 5 Hz. H5'): 4.84 (m, 2H, H4', H5"); 6.15 (m, 2H, H2', H3'); 7.46–8.15 (m, 16H, Ar, H1'); 8.25 (s, 1H. H5); 10.40 (s, 1H, CHO).

Example 5

Preparation of ethyl 1-(b-D-2',3',5'-tri-O-benzoyl-ribofuranosyl)-4-(1-hydroxy-2-nitro)ethyl-pyrazole-3-carboxylate (6). Al_2O_3 (Fluka: type 507 C neutral, 1 g, 9.8 mmol) was added, under argon atmosphere at 0°C, to a solution of 5 (1.2 g, 2 mmol) in nitromethane (0.12 mL, 2.2 mmol). After 12h at room temperature, the mixture was diluted with CH_2Cl_2 (50 mL) filtered and concentrated to dryness to give an oil which was purified by column chromatography (EtOAc/Hexane, 1/1 in presence of Et_3N).

6: syrup; yield 65%; ^{1}H NMR ($CDCl_3$) d 1.42 (t, 3H, J = 7 Hz, Ethyl); 4.47 (q, 2H, Ethyl); 4.60 (dd. 1H, J = 12 and 5 Hz, H5'); 4.67 (m, 3H, OH, $-CH_2NO_2$); 4.84 (m, 2H. H4', H5"); 5.62 (m, 1H, –CHOH–); 6.12 (m. 2H, H2', H3'); 7.70 (m, 17H, Ar, H1', H5)

Example 6

Preparation of (E) and (Z) ethyl 1-(b-D-2',3',5'-tri-O-benzoyl-ribofuranosyl)-4-(2-carboxyethyl)vinyl-pyrazole-3-carboxylate (7a). The solution of carboxyethylmethylene phosphorane (0.62 g, 1.8 mmol) and 5 (0.92 g, 1.5 mmol) in dry toluene (10 mL) was refluxed under argon atmosphere until complete reaction (TLC: EtOAc/Hexane, 3/7). The mixture was evaporated in vacuo to give a crude oil which was purified by column chromatography (EtOAc/Hexane, 3/7). Evaporation of the appropriate fractions gave the expected products.

7a (*E*): syrup, yield 73%: ^{1}H NMR ($CDCl_3$) d 1.30 (m, 6H. Ethyl); 4.20 (q, 2H. J = 7 Hz, Ethyl); 4.50 (q, 2H, J = 7 Hz. Ethyl); 4.63 (dd, 1H, J = 12 and 5 Hz. H5'); 4.81 (m, 2H. H4', H5"); 6.18 (m, 2H. H2', H3'): 6.36 (d, 1H, J = 16 Hz, vinyl); 7.80 (m, 16H, Ar. H1'); 8.12 (d, 1H, J = 16 Hz, vinyl); 8.17 (s. 1H. H5).

7a (*Z*): syrup, yield 5%; ^{1}H NMR ($CDCl_3$) d 1.30 (m, 6H, J = 7 Hz. Ethyl); 4.20 (q, 2H. J = 7 Hz, Ethyl); 4.50 (q, 2H, J = 7 Hz. Ethyl); 4.60 (dd. 1H, J = 12 and 5 Hz, H5'); 4.83 (m, 2H. H4', H5"); 5.91 (d, 1H, J = 12 Hz. vinyl); 6.18 (m, 2H. H2', H3'); 7.61 (d, 1H. J = 12 Hz. vinyl); 7.80 (m, 16H, Ar, H1'); 8.89 (s. 1H. H5).

Example 7

Preparation of (E) and (Z) ethyl 1-(b-D-2',3',5'-tri-O-benzoyl-ribofuranosyl)-4-vinyl and -4-(2-bromo)-vinyl-pyrazole-3-carboxylate (7b,c). nBuLi 1.6 M (2.3 mL, 3.65 mmol) was added. under argon atmosphere at 0°C. to a solution of the appropriate

triphenylphosphonium bromide derivative (3.1 mmol) in dry THF (10 mL). 1h later, a solution of **5** (1.2 g, 2 mmol) in dry THF (10 mL) was added and the red mixture stirred for additional 2h. TLC analysis (EtOAc/Hexane, 1/1) evidenciated complete reaction. The mixture was poured into crushed ice (50 g), extracted with EtOAc (3 x 50 mL), dried and concentrated to dryness to give a crude oil which was purified by column chromatography (EtOAc/Hexane, 1/1). Evaporation of the appropriate fractions gave the expected products.

7b: syrup; yield 53%; ^{1}H NMR ($CDCl_3$) d 1.42 (t, 6H, J = 7 Hz, Ethyl); 4.30 (q, 2H, Ethyl); 4.62 (dd, 1H, J = 12 and 5 Hz, H5'); 4.84 (m, 2H, H4', H5"); 5.23 (dd, 1H, J = 1.4 Hz, 11 Hz, vinyl); 5.54 (dd, 1H, J = 1.4 Hz, 18 Hz, vinyl); 6.09 (m, 2H, H2', H3'); 7.11 (dd, 1H, J = 11 Hz, 18 Hz, vinyl); 7.88 (m, 17H, Ar, H1', H5).

7c(*E*)+(*Z*): syrup; yield 35%; ^{1}H NMR ($CDCl_3$) d 1.40 (m, 12H, Ethyl); 4.32 (m, 4H, Ethyl); 4.60 (m, 2H, H5'); 4.82 (m, 4H, H4', H5"); 6.12 (m, 4H, H2', H3'); 6.69 (d, 1H, J = 8 Hz, (*Z*)-vinyl); 6.69 (d, 1H, J = 14 Hz, (*E*)-vinyl); 7.85 (m, 36H, Ar, H1', H5, (*E*)-vinyl, (*Z*)-vinyl).

Example 8

Preparation of (E) ethyl 1-(b-D-2',3',5'-tri-O-benzoyl-ribofuranosyl)-4-(2-nitro)vinyl-pyrazole-3-carboxylate (7d). The nitro derivative **6** (0.86 g, 1.3 mmol) was dissolved in dry CH_2Cl_2 (10 mL) at 0°C and CH_3SO_2Cl (0.2 mL, 2.6 mmol) was added followed by Et_3N (0.36 mL, 2.6 mmol) drop to drop. After 2 h at room temperature TLC (EtOAc/Hexane, 3/7) indicated complete reaction. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with water (50 mL), 10% citric acid/H_2O (50 mL), water (50 mL) dried and concentrated to dryness to give a residue which was purified by column chromatography (EtOAc/Hexane, 3/7).

7d: syrup ; yield 64%; ^{1}H NMR ($CDCl_3$) d 1.44 (t, 3H, J = 7 Hz, Ethyl); 4.47 (q, 2H, Ethyl); 4.60 (dd, 1H, J = 12 and 5 Hz, H5'); 4.81 (m, 2H, H4', H5"); 6.15 (m, 2H, H2', H3'); 7.66 (d, 1H, J = 14 Hz, vinyl); 7.80 (m, 17H, Ar, 1-H', H5); 8.46 (d, 1H, J = 14 Hz, vinyl).

Example 9

Preparation of 1-tetrahydopyranyl-3-carboxyethylpyrazoles 9 and 10. The starting pyrazoles **8** and **4** (5 mmol) and pTSOH x H_2O (0.1 g, 0.5 mmol) were dissolved in $CHCl_3$ (20 mL) and dihydropyran (DHP, 0.63 mL, 6 mmol) was slowly added at 0°C. After 15 min, the reaction mixture was let to reach room temperature and stirred for an additional 3h (EtOAc/Hexane, 1/1). After this time the mixture was washed with satured $NaHCO_3$/H_2O solution (20 mL), water (2 x 20 mL) and the organic phase was anhydrified and evaporated in

vacuo to give a crude oil which was purified by column chromatography (EtOAc/Hexane, 4/6).

9: oil; yield 83%; ^{1}H NMR, $CDCl_3$: 1.32 (t, 3H, J = 7 Hz, Ethyl); 1.60–2.20 (m, 6H, THP); 3.50 (m, 1H, THP); 4.10 (m, 1H, THP); 4.34 (q, 2H, Ethyl); 5.47 (dd, 1H, J = 2.8 Hz, 12.8 Hz, THP); 7.78 (s, 1H, H5).

10: oil; yield 63%; ^{1}H NMR, $CDCl_3$: 1.32 (t, 3H, J = 7 Hz, Ethyl); 1.60–2.20 (m, 6H, THP); 3.50 (m, 1H, THP); 4.10 (m, 1H, THP); 4.34 (q, 2H, Ethyl); 5.49 (dd, 1H, J = 2.8 Hz, 12.8 Hz, THP); 8.25 (s, 1H, H5); 10.40 (s, 1H, CHO).

Example 10

Preparation of 1-tetrahydopyranyl-3-carboxyethyl-4-[2-(trimethylsilyl)ethynyl] pyrazole (11). Freshly distilled anhydrous Et_3N (20 mL) was vigorously purged with argon for 15 min, and halide **9** (0.46 g, 1.3 mmol) was added followed by (trimethylsilyl)acetylene (0.23 mL, 1.5 mmol), $(Ph_3P)_2PdCl_2$ (0.010 g, 0.023 mmol), and CuI (0.0053 g, 0.027 mmol). This suspension was stirred at room temperature for 1 h and then at 60° C for 2 h under positive argon pressure (TLC, EtOAc/ Hexane, 3:7). When the reaction was completed, the solvent was evaporated, the brown residue was dissolved in dichloromethane (100 mL), washed with 2% disodium EDTA/H_2O (2 x 50 mL) and H_2O (2 x 50 mL), dried, and evaporated. The resulting brown oil was purified by chromatography (EtOAc/ Hexane, 3:7 in presence of traces of Et_3N). Appropriate fractions were evaporated to give **11** as a yellow oil.

11: oil; yield 93%; ^{1}H NMR, $CDCl_3$: 0.24 (s, 9H, SiMe3); 1.32 (t, 3H, J = 7 Hz, Ethyl); 1.60–2.20 (m, 6H, THP); 3.50 (m, 1H, THP); 4.10 (m, 1H, THP); 4.34 (q, 2H, Ethyl); 5.45 (dd, 1H, J = 2.8 Hz, 12.8 Hz, THP); 7.85 (s, 1H, H5).

Example 11

1-tetrahydopyranyl-3-carboxyethyl-4-ethynyl-pyrazole (12). Compound **11** (0.115 g, 0.36 mmol) was dissolved in 20 mL of anhydrous THF and TBAF (0.114 g, 0.36 mmol) was added under stirring at room temperature. After 1h TLC (EtOAc/ Hexane, 3:7) indicated complete reaction, the mixture was evaporated to dryness and the residue brown oil was purified by column chromatography (EtOAc/ Hexane, 3:7). Evaporation of appropriate fractions gave a yellow oil.

12: oil; yield 90%; ^{1}H NMR, $CDCl_3$: 1.32 (t, 3H, J = 7 Hz, Ethyl); 1.60–2.20 (m, 6H, THP); 3.22 (s, 1H, Ethynyl); 3.52 (m, 1H, THP); 4.10 (m, 1H, THP); 4.44 (q, 2H, Ethyl); 5.47 (dd, 1H, J = 2.8 Hz, 12.8 Hz, THP); 7.87 (s, 1H, H5).

Example 12

Preparation of 1-tetrahydopyranyl-3-carboxyethyl-4-(1-hydroxy-2-nitro)ethyl pyrazole (13).

Al_2O_3 (Fluka: typ 507 C neutral, 1 g, 9.8 mmol) was added, under argon atmosphere at 0°C. to a solution of **10** (0.5 g, 2 mmol) in nitromethane (0.12 mL, 2.2 mmol). After 12h at room temperature, the mixture was diluted with CH_2Cl_2 (50 mL), filtered and evaporated in vacuo to give an oil which was purified by column chromatography (EtOAc/Hexane, 1/1).

13: oil; yield 65%; ^1H NMR, $CDCl_3$: 1.42 (t, 3H, J=7 Hz, Ethyl); 1.60–2.20 (m, 6H, THP); 3.50 (m, 1H, THP); 4.10 (m, 1H, THP); 4.47 (q, 2H, Ethyl); 4.67 (m, 3H, OH, $-CH_2NO_2$); 5.49 (dd, 1H, J = 2.8 Hz, 12.8 Hz, THP); 5.62 (m, 1H, -CHOH-); 7.75 (s, 1H, H5).

Example 13

Preparation of 1-tetrahydopyranyl-3-carboxyethyl-4-(*E*)-(2-nitro)vinyl pyrazole (14a). The nitro derivative **13** (0.4 g, 1.3 mmol) was dissolved in CH_2Cl_2 (10 mL) at 0°C and CH_3SO_2Cl (0.2 mL, 2.6 mmol) was added followed by TEA (0.36 mL, 2.6 mmol) drop to drop. After 2 h at room temperature TLC (EtOAc/Hexane, 3/7) indicated complete reaction. The reaction mixture was diluted with CH_2Cl_2 (50 mL) washed with water (50 mL), 10% citric acid/H_2O (50 mL), water (50 mL) anhydrified evaporated in vacuo to give a residue which was purified by column chromatography (EtOAc/Hexane, 3/7).

14a: mp 77–78°C; yield 86%; ^1H NMR, $CDCl_3$: 1.44 (t, 3H, J=7 Hz, Ethyl); 1.60–2.20 (m, 6H, THP); 3.70 (m, 1H, THP); 4.10 (m, 1H, THP); 4.47 (q, 2H, Ethyl); 5.49 (dd, 1H, J = 2.8 Hz, 12.8 Hz, THP); 7.66 (d, 1H, J=14 Hz, vinyl); 8.46 (d, 1H, J=14 Hz, vinyl); 8.07 (s, 1H, H5).

Example 14

Preparation of (E) and (Z) 1-tetrahydopyranyl-3-carboxyethyl-4-(2-carboxyethyl)vinyl pyrazole (14b). A solution of carboxyethylmethylene phosphorane (0.62 g, 1.8 mmol) and the aldehyde **5** (0.37 g, 1.5 mmol) in anhydrous toluene (10 mL) under argon atmosphere was heated at reflux conditions until complete reaction (TLC: EtOAc/Hexane, 3/7). The mixture was evaporated in vacuo to give a crude oil which was purified by column chromatography (EtOAc/Hexane, 3/7). Evaporation of the appropriate fractions gave the expected product.

14b (*E*): oil, yield 73%: ^1H NMR, $CDCl_3$: 1.30 (m, 6H, Ethyl); 1.70–2.0 (m, 6H, THP); 3.70 (m, 1H, THP); 4.10–4.30 (m, 1H, THP; m, 2H, Ethyl); 4.50 (q, 2H, J=7 Hz, Ethyl); 5.50 (dd,

1H, J = 2.8 Hz, 12.8 Hz, THP); 6.36 (d, 1H, J = 16 Hz, vinyl); 8.12 (d, 1H, J = 16 Hz, vinyl); 8.17 (s, 1H, H5).

14b (*Z*): oil, yield 5%; ^{1}H NMR, $CDCl_3$: 1.30 (m, 6H, J=7 Hz, Ethyl); 1.70–2.0 (m, 6H, THP); 3.70 (m, 1H, THP); 4.10–4.30 (m, 1H, THP; m, 2H, Ethyl); 4.50 (q, 2H, J=7 Hz, Ethyl); 5.50 (dd, 1H, J = 2.8 Hz, 12.8 Hz, THP); 5.91 (d, 1H, J = 12 Hz, vinyl); 7.61 (d, 1H, J = 12 Hz, vinyl); 8.89 (s, 1H, H5).

Example 15

Preparation of 1-tetrahydopyranyl-3-carboxyethyl-4-vinyl and -(2-bromo)vinyl pyrazole (14c,d). nBuLi 1.6 M (4.6 mL, 7.3 mmol) was added, under argon atmosphere at 0°C, to a solution of the appropriate triphenylphosphonium bromide derivative (6.2 mmol) in anhydrous THF (10 mL). After 1h, the aldehyde **5** (1 g, 4 mmol) was added and the red solution was stirred for additional 2 h. TLC analysis (EtOAc/Hexane, 1/1) evidentiated complete reaction. The mixture was poured into crushed ice (100 g), extracted with EtOAc (3 x 50 mL), anhydrified and evaporated in vacuo to give a crude oil which was purified by column chromatography. Evaporation of the appropriate fractions gave the expected products:

14c: oil; yield 62%; ^{1}H NMR, $CDCl_3$: 1.40 (t, 6H, J=7 Hz, Ethyl); 1.70–2.0 (m, 6H, THP); 3.70 (m, 1H, THP); 4.10 (m, 1H, THP); 4.30 (q, 2H, Ethyl); 5.21 (dd, 1H, J = 1.4 Hz, 11 Hz, vinyl); 5.48 (dd, 1H, J = 2.8 Hz, 12.8 Hz, THP); 5.54 (dd, 1H, J = 1.4 Hz, 18 Hz, vinyl); 7.11 (dd, 1H, J = 11 Hz, 18 Hz); 7.83 (s, 1H, H5).

14d: oil; yield 50%; (*E*) + (*Z*) mixture: yield 50%; ^{1}H NMR, $CDCl_3$: 1.40 (m, 6H, Ethyl); 1.70–2.0 (m, 12H, THP); 3.70 (m, 2H, THP); 4.10 (m, 2H, THP); 4.41 (m, 4H, Ethyl); 5.49 (m, 2H, THP); 6.38 (d, 1H, J = 8 Hz, (*Z*)–vinyl); 6.69 (d, 1H, J = 14 Hz, (*E*)–vinyl); 7.52 (d, 1H, J = 14 Hz, (*E*)–vinyl); 7.80 (d, 1H, J = 8 Hz, (*Z*)–vinyl); 7.19 (s, 1H, H5, (*Z*)–isomer); 7.61 (s, 1H, H5, (*E*)–*i*somer).

Example 16

Synthesis of 1-b-D-ribofuranosyl-4-ethynyl-pyrazole-3-carboxamide (15). Compound **3** (0.2 g, 0.317 mmol) was dissolved at 4°C in 20 mL of methanolic ammonia (saturated at –15°C) and let to stir, in a firmly capped flask, at 4°C for 24 h. After this time TLC (CH_2Cl_2/MeOH, 8/2) indicated complete reaction and the mixture was evaporated to dryness. The residue was treated with ether (3 X 50 mL) and the insoluble gum was purified by column chromatography (CH_2Cl_2/MeOH, 8/2). Evaporation of the appropriate fractions gave a yellow syrup.

15: syrup; yield 82%; UV (MeOH) nm l_{max} 207 (e 16300), 230 (e 7000); $l_{shoulder}$ 254 (e 3000); l_{min} 221 (e 6500); ^{1}H NMR, (DMSO-d_6) d 3.30-3.60 (m, 2H, H5',5"); 3.90 (m, 1H, H4'); 4.20 (m, 1H, H3'); 4.35 (m, 1H, H2'); 5.02 (s, 1H, ethynyl); 5.05 (t, 1H, J = 5.5 Hz, OH); 5.24 (d, 1H, J = 5.4 Hz, OH); 5.52 (d, 1H, J = 5.8 Hz, OH); 5.66 (d, 1H, J = 4.3 Hz, H1'); 7.20(br s, 1H, $CONH_2$); 7.40 (br s, 1H, $CONH_2$); 8.23 (s, 1H, H5).

Example 17

General procedure for deprotection of derivatives 5, 7a-d. The removal of protective benzoyl groups of compounds **5, 7a-d** (2.5 mmol) was carried out as described for compound **15.** After the usual work-up, the residue was purified by column chromatography (CH_2Cl_2/MeOH, 8/2). The solid products were crystallized by methanol with diffusion of Et_2O.

16: white solid; yield 83%; mp 107°C; UV (MeOH) nm l_{max} 209 (e 14900), 245 (e 7000); l_{min} 233 (e 6900), 200 (e 8400); ^{1}H NMR, (DMSO-d_6) d 3.52 (m, 2H, H5',5''); 3.94 (m, 1H, H4'); 4.14 (m, 1H, H3'); 4.33 (m, 1H, H2'); 4.99 (t, 1H, J = 5.5 Hz, OH); 5.18 (d, 1H, J = 5.4 Hz, OH); 5.58 (d, 1H, J = 5.8 Hz, OH); 5.74 (d, 1H, J = 4.0 Hz, H1'); 7.68 (sbr, 1H, $CONH_2$); 7.94 (sbr, 1H, $CONH_2$); 8.73 (s, 1H, H5); 10.2 (s, 1H, CHO).

17a: pale yellow solid; yield 77%; mp 97-98°C; UV (MeOH) nm l_{max} 204 (e 15300), 285 (e 16500); $l_{shoulder}$ 224 (e 12200), l_{min} 247 (e 7300); ^{1}H NMR, (DMSO-d_6) d 1.23 (t, 3H, J = 7 Hz, Ethyl); 3.52 (m, 2H, H5',5''); 3.94 (m, 1H, H4'); 4.15 (q, 2H, Ethyl); 4.17 (m, 1H, H3'); 4.33 (m, 1H, H2'); 4.92 (t, 1H, J = 5.5 Hz, OH); 5.13 (d, 1H, J = 5.4 Hz, OH); 5.50 (d, 1H, J = 5.8 Hz, OH); 5.64 (d, 1H, J = 4.4 Hz, H1'); 6.43 (d, 1H, J = 16 Hz, vinyl); 7.43 (sbr, 1H, $CONH_2$); 7.59 (sbr, 1H, $CONH_2$); 8.10 (d, 1H, J = 16 Hz, vinyl); 8.65 (s, 1H, H5).

17b: syrup; yield 68%; UV (MeOH) nm l_{max} 213 (e 10800), 244 (e 8000); l_{min} 235 (e 7900), 200 (e 8400); ^{1}H NMR, (DMSO-d_6) d 3.52 (m, 2H, H5',5''); 3.90 (m, 1H, H4'); 4.17 (m, 1H, H3'); 4.33 (m, 1H, H2'); 4.97 (t, 1H, J = 5.5 Hz, OH); 5.14 (d, 1H, J = 5.4 Hz, OH); 5.29 (dd, 1H, J = 1.4 Hz, 11 Hz, vinyl); 5.55 (d, 1H, J = 5.8 Hz, OH); 5.66 (dd, 1H, J = 1.4 Hz, 18 Hz, vinyl); 5.76 (d, 1H, J = 4.4 Hz, H1'); 7.11 (dd, 1H, J = 11 Hz, 18 Hz, vinyl); 7.40 (sbr, 1H, $CONH_2$); 7.60 (sbr, 1H, $CONH_2$); 8.87 (s, 1H, H5).

17c (Z): mp 67°C; yield 32%; UV (MeOH) nm l_{max} 232 (e 11000), 298 (e 10000); $l_{shoulder}$ 345 (e 7500); l_{min} 265 (e 8000); ^{1}H NMR, (DMSO-d_6) d 3.50 (m, 2H, H5',5''); 3.92 (m, 1H, H4'); 4.15 (m, 1H, H3'); 4.30 (m, 1H, H2'); 4.96 (t, 1H, J = 5.5 Hz, OH); 5.15 (d, 1H, J = 5.4 Hz, OH); 5.53 (d, 1H, J = 5.8 Hz, OH); 5.75 (d, 1H, J = 4.4 Hz, H1'); 6.56 (d, 1H, J = 8 Hz,

(Z)-vinyl); 7.40 (sbr. 1H, NH); 7.56 (d, 1H. J = 8 Hz, (Z)-vinyl); 7.60 (sbr, 1H, NH); 8.87 (s, 1H. H5).

17c (E): syrup; yield 33%: UV (MeOH) nm l_{max} 230 (e 11500), 300 (9700); $l_{shoulder}$ 350 (e 7000): lmin 265 (e 8500); ^{1}H NMR. (DMSO-d6) d 3.51 (m, 2H, H5',5''); 3.90 (m, 1H, H4'); 4.12 (m, 1H, H3'); 4.28 (m, 1H, H2'): 4.94 (t, 1H, J = 5.5 Hz, OH); 5.18 (d, 1H, J = 5.4 Hz, OH): 5.51 (d, 1H, J = 5.8 Hz, OH); 5.76 (d, 1H, J = 4.4 Hz, H1'); 6.69 (d, 1H. J = 14 Hz, (E)-vinyl): 7.45 (sbr, 1H. NH); 7.52 (d, 1H. J = 14 Hz, (E)-vinyl); 7.63 (sbr, 1H, NH); 8.97 (s, 1H, H5).

17d: unstable; syrup; yield 28%; UV (MeOH) nm l_{max} 205 (e 10500), 235 (e 7900); 315 (e 11000), l_{min} 225 (e 7300), 265 (e 3500); ^{1}H NMR, (DMSO-d6) d 3.52 (m, 2H, H5',5''); 3.86 (m, 1H. H4'); 4.14 (m, 1H, H3'); 4.30 (m, 1H, H2'); 4.94 (t, 1H, J = 5.5 Hz, OH); 5.15 (d, 1H, J = 5.4 Hz, OH); 5.53 (d, 1H, J = 5.8 Hz. OH); 5.74 (d, 1H, J = 4.4 Hz, H1'); 7.70 (d, 1H, J = 14 Hz. vinyl); 8.10 (s, 1H. H5); 8.40 (d, 1H, J = 14 Hz, vinyl); 7.40 (sbr, 1H. $CONH_2$); 7.60 (sbr. 1H, $CONH_2$); 8.85 (s, 1H, H5).

Example 18

Preparation of 4-iodo-3-(3'-methyl-1',2',4'-oxadiazolyl)-1-b-D-ribofuranosyl pyrazole (18). A mixture of acetamidoxime (0.244 g, 3.3 mmol), NaH (0.87 g, 3.63 mmol) and powdered molecular sieve (type 4 Å, 0.15 g) in dry THF (15 mL) was warmed at 60°C under argon atmosphere. After 1 h. a solution of **1** (0.25 g, 0.36 mmol) in dry THF (10 mL) was added and the mixture was refluxed for an additional 2 h. The mixture was then filtered and evaporated to dryness and the residue purified by column chromatography (CH_2Cl_2/MeOH, 9/1). Evaporation of the appropriate fractions gave compound **18** as a white powder.

18: yield 37%; UV (MeOH) l_{max} 205 (e 16400), 242 (e 8600); $l_{shoulder}$ 270 (e 4 500); l_{min} 227 (e 7000); ^{1}H NMR, (DMSO-d6) d 2.43 (s, 3H, Me): 3.50 (m, 2H, H5', 5''): 3.96 (m. 1H, H4'): 4.11 (m, 1H, H3'); 4.32 (m. 1H. H2'); 5.10 (t, 1H. J = 5.5 Hz, OH); 5.20 (d. 1H. J = 5.3 Hz. OH); 5.60 (d, 1H. J = 5.9 Hz, OH): 5.86 (d, 1H. J = 4.2 Hz, H1'); 8.50 (s. 1H. H5).

Example 19

Preparation of 4-iodo-1-b-D-ribofuranosylpyrazole-3-carboxylic acid (19). A solution of 2 (0.264 g, 0.687 mmol) in 0.35 M NaOH (5 mL) was stirred at room temperature for 5 h. The solution was then neutralized with Dowex (50W H^+), filtered and the pad washed with

water (3 x 5 mL). Evaporation to dryness of the collected fractions gave a yellow syrup which was crystallized from EtOH absolute to give a white solid.

19: yield 94%; mp 138–140 C°; UV (MeOH) l_{max} 205 (e 19400), $l_{shoulder}$ 240 (e 6100); ^1H NMR, (DMSO–d_6) d 3.50 (m, 2H, H5', 5") ; 3.88 (m, 1H, H4'); 4.08 (m, 1H, H3'); 4.25 (m, 1H, H2'); 5.00 (br, 1H, OH); 5.30 (br, 1H, OH); 5.60 (br, 1H, OH); 5.69 (d, 1H, J = 4.2 Hz, H1'); 8.06 (s, 1H, H5); 10.00 (br, 1H, COOH).

Example 20

Preparation of methyl 1–(b–D–2',3',5'–tri–O–terbutyldimethylsilyl–ribofuranosyl)–4–iodo–pyrazole–3–carboxylate (20). TBDSCl (0.434 g, 2.74 mmol) imidazole (0.195 g, 2.74 mmol) and DMAP (0.015 gr, 0.13 mmol) were added, under argon atmosphere, to a solution of **2** (0.2 gr, 0.52 mmol) in dry DMF (5 mL),. After 48 h at room temperature, the reaction was completed (TLC EtOAc/Hexane, 3/7). The mixture was evaporated to dryness and the residue purified by column chromatography (EtOAc/Hexane, 3/7) to give a pale yellow oil.

20: oil; yield 85%. ^1H NMR ($CDCl_3$) d 0.12–0.31 (6s, 18H, 6xSi–Me_2); 0.98–1.11 (3s, 27H, Si–tBut); 3.90 (dd, 1H, $J_{5'-5"}$ = 11.6 Hz, $J_{4'-5'}$ = 2.3 Hz, H5'); 4.06 (dd, 1H, H5"); 4.08 (s, 3H, OMe); 4.24 (m, 1H, H4'); 4.35 (m, 1H, H3'); 4.51 (m, 1H, H2'); 5.96 (d, 1H, J = 4.8 Hz, H1'); 8.15 (s, 1H, H5).

Example 21

Preparation of butyl 4–iodo–1–b–D–ribofuranosylpyrazole–3–carboxylate (21). Dry butyl alcohol (0.25 mL, 0.19 g, 2.6 mmol) and 80% NaH (0.016 g, 0.066 mmol) were added to a solution of **20** (0.80 g, 0.11 mmol) in dry benzene (2 mL) and the mixture was stirred for 1 h at room temperature under argon atmosphere. The mixure was then diluted with EtOAc (5 mL) and then washed with cold 5% HCl (2 mL), dried and evaporated to dryness to give a crude residue in nearly quantitative yield. A sample of crude oil was purified by column chromatography (EtOAc/Hexane, 1/9).^1H NMR ($CDCl_3$) d 0.11–0.28 (7s, 21H, 6xSi–Me_2 and Me); 0.97–1.08 (3s, 27H, Si–tBut); 1.59–1.90 (m, 4H, butyl); 3.85–4.10(m, 2H, H5' and H5"); 4.22 (m, 1H, H4'); 4.35 (m, 1H, H3'); 4.50 (m, 3H, H2' and butyl); 5.94 (d, 1H, J = 4.8 Hz, H1'); 8.10 (s, 1H, H5).

The above described crude material (0.1 g) was dissolved in dry methanol (10 mL) and then glacial acetic acid (26 mL, 0.46 mmol), followed by NH_4F (0.058 g, 1.56 mmol), were added. The reaction mixture was heated at reflux conditions and under argon positive pressure for 12 h. After this time TLC (CH_2Cl_2/MeOH, 9/1) indicated complete reaction. The mixture was

evaporated to dryness and co-evaporated with EtOH (2 x 20 mL). The resulting residue was purified by column chromatography (CH_2Cl_2/MeOH, 9/1) to give a pale yellow syrup.

21: syrup; yield 85%. UV (MeOH) l_{max} 259 (e 3100), l_{min} 242 (e 2800); ^1H NMR, ($CDCl_3$) d 0.94 (t, 3H, J = 7 Hz, butyl); 1.5 (m. 2H, butyl); 1.7 (m, 2H, butyl); 3.56 (m, 2H, H5', 5") ; 4.2 (m, 1H, H4'); 4.31 (t, 2H, J = 6 Hz, butyl); 4.5 (m, 1H, H3'); 4.6 (br, 3H, H2', OH x 2); 5.00 (br, 1H, OH); 5.79 (d, 1H, J = 3.5 Hz. H1'); 7.84 (s, 1H, H5).

Example 22

Preparation of 4-iodo-1-b-D-ribofuranosyl-pyrazole-3-carboxamide (22). The protected compound 1 (0.5 g, 0.76 mmol) was dissolved in 20 mL of methanolic ammonia (saturated at -15 °C) and let to stir overnight in a firmly capped flask at 4 °C. After this time. TLC (CH_2Cl_2/MeOH, 9/1) indicated complete reaction and the mixture was evaporated to dryness. The residue was triturated with ether (3 x 20 mL) and the insoluble solid was purified by column chromatography (CH_2Cl_2/MeOH, 9/1). After evaporation of the appropriate fractions, the resiude was crystallized by methanol with diffusion of Et_2O.

22: solid, mp 181-184 °C; yield 74%; UV (MeOH) nm l_{max} 205 (e 14800), 257 (e 2800); l_{min} 246 (e 2700); ^1H NMR, (DMSO-d_6) d 3.40-3-70 (m, 2H, H5',5"); 3.92 (m, 1H, H4'); 4.11 (m, 1H. H3'); 4.31 (m, 1H. H2'); 4.95 (t, 1H, J = 5.5 Hz, OH); 5.14 (d, 1H, J = 5.4 Hz, OH); 5.49 (d. 1H, J = 5.8 Hz. OH); 5.65 (d. 1H, J = 4.3 Hz. H1'); 7.32 (br s, 1H, $CONH_2$); 7.52 (br s, 1H. $CONH_2$); 8.26 (s, 1H. H5).

Example 23

Preparation of methyl 4-iodo-1-b-D-ribofuranosyl-pyrazole-3-carboxylate 5'-O-monophosphate disodium salt (23) and 4-iodo-1-b-D-ribofuranosyl-pyrazole-3-carboxamide 5'-O-monophosphate disodium salt (24). The deprotected nucleosides 2 or 22 were added (0.271 mmol) to a mixture of freshly distilled phosphoryl chloride (217 mL, 2.38 mmol), water (27 mL, 1.52 mmol), pyridine (210 mL, 2.6 mmol) and acetonitrile (300 mL, 5.5 mmol). The mixture was mantained at 0 °C for 4 h under vigourous stirring, poured into ice water, further stirred for 1 h at 5 °C and then stored overnight at 5° C. TLC (isopropanole/ H_2O/ NH_4OH. 11/ 2/ 7) showed complete transformation of nucleosides to nucleotides. Aqueous solvent was evaporated under vacuum at 40° C; the crude material was eluted through a column of anionic exchange resin Sephadex-DEAE, A-25 using a linear gradient of ammonium formiate buffer as eluent (0.25 to 0.8 M, pH 7.4), purified by HPLC and finally

filtered through a short column of cationic exchange resin Dowex 50Wx2-200, Na^+ form, to give the disodium salt of the desired nucleotides after liophylisation.

23: white solid, mp 162-164° C, yield 40%. UV (H_2O) nm l_{max} 258 (e 2800); l_{min} 245 (e 2500); ^1H NMR, (DMSO-d_6 + D_2O) d 3.78 (s, 3H, OMe); 3.74-4.36 (m, 5H, H5',5", H4', H3' and H2'); 5.63 (d, 1H, J = 5.2 Hz, H1'); 8.36 (s, 1H, H5). ^{31}P NMR, (DMSO-d_6 + D_2O) d 0.17. FAB MS (>0, G-T) m/e 509 $(M+H)^+$.

24: white solid, mp XX °C, yield 63%. UV (H_2O) nm l_{max} 258 (e 2800); $l_{shoulder}$ 245; ^1H NMR, (DMSO-d_6) d 3.75 (m, 2H, H5',5"); 4.00 (m, 1H, H4'); 4.24-4.50 (m, 4H, H3', H2', OH2' and OH3'); 5.62 (d, 1H, J = 4.9 Hz, H1'); 7.22 (br s, 1H, $CONH_2$); 7.72 (br s, 1H, $CONH_2$); 8.22 (s, 1H, H5). ^{31}P NMR, (DMSO-d_6 + D_2O) d 1.7. FAB MS (>0, G-T) m/e 494 $(M+H)^+$.

Example 24

Preparation of methyl 4-iodo-1-b-D-ribofuranosyl-pyrazole-3-carboxylate-5'-yl bis[(pivaloyloxy)methyl] phosphate (25) and 4-iodo-1-b-D-ribofuranosyl-pyrazole-3-carboxamide-5'-yl bis[(pivaloyloxy)methyl] phosphate (26). Bis[(pivaloyloxy)methyl] hydrogen phosphate (0.49 g, 1.5 mmol), compound **2** or **22** (1 mmol), and triphenylphosphine (0.39 g, 1.5 mmol) were dissolved in dimethylacetamide (5 mL). A solution of diethyl azodicarboxylate (0.26 g, 1.5 mmol) in dimethylacetamide (3 mL) was slowly dropped with stirring, and the reaction mixture was heated at 60 °C for 5 days under an argon atmosphere. The dimethylacetamide was evaporated under reduced pressure. Toluene (25 mL) was added and the solution was again evaporated. This procedure was repeated twice. The crude material were maintained under high vacuum for 24 h to remove residual dimethylacetamide. The remaining product was chromatographed on a column silica using CH_2Cl_2/MeOH (9.5/0.5) as eluent. The compound was isolated as colorless compound.

25: oil, yield 57 %.

26: oil, yield 52 %.

Example 25

Preparation of methyl 4-iodo-1-(b-D-2',3'-O-isopropylidene-ribofuranosyl)-pyrazole-3-carboxylate (27) and 4-iodo-1-(b-D-2',3'-O-isopropylidene-ribofuranosyl)-pyrazole-3-carboxamide (28). 2,2-dimethoxypropane (0.71 mL, 5.8 mmol) and bis-(p-nitrophenyl)-phosphate (30 mg, 0.09 mmol) were added to a solution of **2** or **22** (1 mmol) in anhydrous acetone (10 mL) and the mixture was let to stir at room temperature. 3 h

later, an aqueous solution of NH_4HCO_3 0.25 M (50 mL) was added at 0 °C, the solvent was evaporated under reduced pressure and the crude material was purified by flash chromatography (Hexane/AcOEt, 7/3 in presence of some drop of triethylamine).

27: pale yellow oil, yield 68%; ^1H NMR, (CDCl3) d 1.35 (s, 3H, CH_3); 1.58 (s, 3H, CH_3); 3.75 (m, 2H, H5',5"); 3.93 (m, 4H, OMe and H4'); 4.55 (br s, 1H, OH); 5.07 (m, 2H, H2' and H3'); 5.86 (d, 1H, J = 2.2 Hz, H1'); 7.74 (s, 1H, H5).

28: pale yellow oil, yield 60%.

Example 26

Preparation of methyl 4-iodo-1-(b-D-2',3'-O-isopropylidene-ribofuranosyl)-pyrazole-3-carboxylate-5'yl bis(S-pivaloyl-2-thioethyl) phosphate (29) and 4-iodo-1-(b-D-2',3'-O-isopropylidene-ribofuranosyl)-pyrazole-3-carboxamide-5'-yl bis(S-pivaloyl-2-thioethyl) phosphate (30). 1H-tetrazole (0.21 g, 3.0 mmol) was added to a stirred solution of the protected derivatives **27** or **28** (1.0 mmol) and the bis(S-pivaloyl-2-ethioethyl) N,N-diisopropylphosphoramidite (1.2 mmol) in anhydrous THF (2 mL) at room temperature. After 30 min, the reaction mixture was cooled at -40° C, a solution of tert-butyl-hydroperoxide (1.3 mmol) in dichloromethane was added and then the mixture was allowed to warm to room temperature over 1 h. Sodium sulfite (10% solution, 1.3 mL) was added to destroy the excess t-butyl-hydroperoxide, the organic layer was separated and the aqueous layer was washed with dichloromethane (2 x 10 mL). The combined organic layers were first washed with saturated aqueous sodium hydrogen carbonate (5 mL) and then with water (3 x 5 mL), dried over sodium sulfate, filtered, and evaporated to dryness under reduced pressure. Column chromatography of the residue on silica gel afforded the title compounds as pale yellow oils.

29: pale yellow syrup, yield 43%; ^1H NMR, ($CDCl_3$) δ 1.22 (s, 18H, 2 x t-Bu); 1.36 (s, 3H. CH_3); 1.66 (s, 3H. CH_3); 3.07-3.16 (m, 4H, 2 x CH_2S); 3.93 (m, 3H, OMe); 4.01-4.14 (m. 6H, 2 x CH_2O, H5',5"); 4.50 (m, 1H, H4'): 4.95 (m, 1H. H3'); 5.26 (m, 1H, H2'); 5.96 (d, 1H. J = 1.3 Hz, H1'); 7.81 (s, 1H, H5). ^{31}P, ($CDCl_3$) δ -1.23.

30: colourless syrup, yield 45%; ^1H NMR, ($CDCl_3$) δ 1.22 and 1.23 (s, 18H, 2 x t-Bu); 1.37 (s, 3H. CH_3); 1.57 (s, 3H. CH_3); 3.08 and 3.14 (t, 4H, J = 6.8 Hz, 2 x CH_2S); 3.96-4.40 (m. 6H, 2 x CH_2O, H5',5"); 4.50 (m, 1H, H4'); 5.08 (m, 1H, H3'); 5.18 (m, 1H, H2'); 5.55 (sbr, 1H. NH_2); 5.92 (d, 1H. J = 1.0 Hz, H1'); 7.50 (sbr, 1H, NH_2); 7.71 (s, 1H, H5). ^{31}P, ($CDCl_3$) δ -0.75.

Example 27

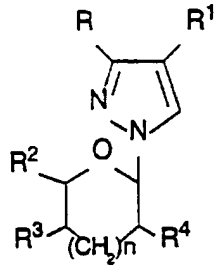
Preparation of methyl 4-iodo-1-β-D-ribofuranosyl-pyrazole-3-carboxylate-5'yl bis(S-pivaloyl-2-thioethyl) phosphate (31) and 4-iodo-1-β-D-ribofuranosyl-pyrazole-3-carboxamide-5'yl bis(S-pivaloyl-2-thioethyl) phosphate (32). Protected derivatives **29** and **30** (0.05 mmol) were dissolved in methanol (5 mL) and TsOH (38 mg, 0.2 mmol) was added. The mixture was let to stirr overnight at 60° C. TLC (CH_2Cl_2:MeOH/9.5:0.5) showed complete reaction. After purification on column chromatography (eluent 4% methanol in CH_2Cl_2) the espected products were recovered.

31: colourless syrup, yield 94%; ^1H NMR, ($CDCl_3$) δ 1.21 and 1.23 (s, 18H, 2 x t-Bu); 2.02 (sbr, 2H. 2 x OH); 3.09–3.17 (m, 4H, 2 x CH_2S); 3.93 (m, 3H, OMe); 4.05–4.17 (m, 4H. 2 x CH_2O): 4.29–4.36 (m, 3H, H4', H5',5"); 4.40–4.54 (m, 2H, H2', H3'); 5.88 (d, 1H. J = 2.2 Hz, H1'); 7.88 (s, 1H, H5). ^{31}P, ($CDCl_3$) δ -1.02.

30: white foam, yield 80%; ^1H NMR, ($CDCl_3$) δ 1.21 and 1.23 (s, 18H, 2 x t-Bu); 1.96 (sbr, 1H, OH); 3.04 and 3.13 (t, 4H, J = 6.8 Hz, 2 x CH_2S); 3.48 (sbr, 1H, OH); 4.00 and 4.14 (q, 4H, J = 7 Hz, 2 x CH_2O); 4.20–4.28 (m, 2H, H5',5"); 4.48–4.58 (m, 3H, H2", H3', H4'); 5.77 (d, 1H, J = 2.4 Hz, H1'); 5.92 (sbr, 1H. NH_2); 7.39 (sbr, 1H, NH_2); 7.74 (s, 1H, H5). ^{31}P, ($CDCl_3$) δ -1.43.

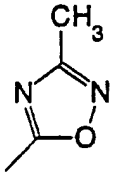
CLAIMS

1. Compounds of general formula (I)



(I)

wherein R is COOH, COOR',



and R' is a $C_1 - C_5$ alkyl radical

R^1 is I, Br, Cl, F, CF_3, CN, SCN, CHO, $CH=CH_2$, CH=CH Br, $C\equiv C-Si(CH_3)_3$, CH=CH $COOCH_2CH_3$, $C\equiv CH$, $CH_2=CH\,NO_2$,

n is 0 or 1,

R^2 is H, CH_2OH, CH_2 OCOPh, CH_2O $Si(CH_3)_2C(CH_3)_3$, $CH_2OPO_3Na_2$, $CH_2OPO[OCH_2O-COC(CH_3)_3]_2$, $CH_2OPO[O\,CH_2\,CH_2\,SCOC(CH_3)_3]_2$,

R^3 and R^4 equal or different from eachother are H, OH, CH_2 OCOPh, CH_2O $Si(CH_3)_2C(CH_3)_3$, or they form together an isopropylidene group,

provided that when $R^2 = R^3 = R^4 = H$, n is always 1.

and with the exclusion of the compound having $R=COOCH_3$, R^1 =I, n=0, $R^3 = CH_2$ OCOPh. $R^3 = R^4$=OCOPh

2. The compound as claimed in claim 1 selected from the group consisting of:

- compound of formula (I) wherein $R = COOCH_3$, R^1 = I, n = 0, $R^2 = CH_2OH$, $R^3 = R^4$ =OH,
- compound of formula (I) wherein $R = COOCH_3$, $R^1 = C\equiv C-Si(CH_3)_3$, n = 0, R^2 = CH_2OCOPh, $R^3 = R^4$= OCOPh,
- compound of formula (I) wherein $R = COOCH_2CH_3$, R^1 = CHO, n=0, $R^2 = CH_2OCOPh$, R^3 = R^4= OCOPh.

- compound of formula (I) wherein R = $COOCH_2CH_3$, R^1 = CH=CH $COOCH_2CH_3$, n =0, R^2 = CH_2OCOPh, $R^3 = R^4$= OCOPh,
- compound of formula (I) wherein R = $COOCH_2CH_3$, $R^1 = CH_2$=CH, n=0, $R^2 = CH_2OCOPh$, $R^3 = R^4$= OCOPh,
- compound of formula (I) wherein R = $COOCH_2CH_3$, R^1 = CH=CHBr, n=0, R^2 = CH_2OCOPh, $R^3 = R^4$= OCOPh,
- compound of formula (I) wherein R = $COOCH_2CH_3$, R^1 = $CH=CHNO_2$, n=0, R^2 = CH_2OCOPh, $R^3 = R^4$= OCOPh,
- compound of formula (I) wherein R = $COOCH_2CH_3$, R^1 = I, n= 1, $R^2 = R^3 = R^4$ = H,
- compound of formula (I) wherein R = $COOCH_2CH_3$, R^1 = CHO, n= 1, $R^2 = R^3 = R^4$ = H,
- compound of formula (I) wherein R = $COOCH_2CH_3$, R^1 = C≡C–Si $(CH_3)_3$, n = 1, $R^2 = R^3 = R^4$ = H,

n = 1, $R^2 = R^3 = R^4$ = H,

- compound of formula (I) wherein R = $COOCH_2CH_3$, R^1 = C≡CH, n = 1, $R^2 = R^3 = R^4$ = H,
- compound of formula (I) wherein R = $COOCH_2CH_3$, R^1 = $CH=CHNO_2$, n = 1, $R^2 = R^3 = R^4$ = H,
- compound of formula (I) wherein R = $COOCH_2CH_3$, R^1 = CH=CH $COOCH_2CH_3$, n = 1, $R^2 = R^3 = R^4$ = H,
- compound of formula (I) wherein R = $COOCH_2CH_3$, $R^1 = CH_2$=CH, n = 1, $R^2 = R^3 = R^4$ = H,
- compound of formula (I) wherein R = $COOCH_2CH_3$, R^1 = CHBr=CH, n = 1, $R^2 = R^3 = R^4$ = H,
- compound of formula (I) wherein R = 3–methyl–1',2',4'–oxadiazol–5'yl, R^1 =I, n = 0, R^2 = CH_2OH, $R^3 = R^4$ =OH,
- compound of formula (I) wherein R = COOH, R^1 = I, n = 0, $R^2 = CH_2OH$, $R^3 = R^4$ =OH,
- compound of formula (I) wherein R = $COOCH_3$, R^1 = I, n = 0,

$R^2 = CH_2O$ $Si(CH_3)_2C(CH_3)_3$, $R^3 = R^4$ = O $Si(CH_3)_2C(CH_3)_3$

- compound of formula (I) wherein R =$COOCH_2CH_2CH_2CH_3$, R^1 = I, n = 0, $R^2 = CH_2OH$, $R^3 = R^4$ =OH,
- compound of formula (I) wherein R = $COOCH_3$, R^1 = I, n = 0, $R^2 = CH_2OPO_3Na_2$, $R^3 = R^4$ =OH,
- compound of formula (I) wherein R = $COOCH_3$, R^1 = I, n = 0,

$R^2 = CH_2OPO[OCH_2O-COC(CH_3)_3]_2$

– compound of formula (I) wherein R = $COOCH_3$, R^1 = I, n = 0, $R^2 = CH_2OH$, R^3 and R4 form together an isopropylidene group,

– compound of formula (I) wherein R = $COOCH_3$, R^1 = I, n = 0,

$R^2 = CH_2OPO[O\ CH_2\ CH_2\ SCOC(CH_3)_3]_2$, R^3 and R4 form together an isopropylidene chain,

– compound of formula (I) wherein R = $COOCH_3$, R^1 = I, n = 0, $R^2 = CH_2OPO[O\ CH_2\ CH_2\ SCOC(CH_3)_3]_2$, $R^3 = R^4 = OH$.

3. A therapeutical composition containing as the active principle at least one compound as claimed in anyone of claims 1 and 2, in combination with suitable excipients or diluents.

4. The therapeutical composition according to claim 3 for the treatment of solid and liquid tumours.

5. The therapeutical composition according to claim 4 wherein said solid tumours are selected from the group consisting of : carcinoma, neuroblastoma, adenocarcinoma, and said liquid tumours are selected from the group consisting of leukemia and lynphoma.

6. The therapeutical composition according to anyone of claims 4 and 5 containing as the active principle the compound of formula (I) wherein R = $COOCH_3$, R^1 = I, n = 0, $R^2 = CH_2OH$. $R^3 = R^4 = OH$.

7. The therapeutical composition according to anyone of claims 4 and 5 containing as the active principle at least one active ingredient selected from the group consisting of :

– compound of formula (I) wherein R = $COOCH_2\ CH_3$, $R^1 = CH=CHNO_2$, n = 1, $R^2 = R^3 = R^4 = H$,

– compound of formula (I) wherein R = $COOCH_2\ CH_3$, $R^1 = CH=CHBr$, n = 1, $R^2 = R^3 = R^4 = H$,

8. The therapeutical composition according to anyone of claims 4–8 further containing other antitumoral agents.

9. The therapeutical composition according to claim 8 wherein said antitumoral agents are selected from the group consisting of tiazofurin, selenazofurin, ribavirin, 6–mercaptopurin, methothrexate,5–fluorouracyl, 6–thioguanine and taxol.

10. The therapeutical composition according to claim 3 further containing as the active ingredient an antiviral agent.

11. The therapeutical composition according to claim 10, wherein said antiviral agents are selected from the group consisting of 2',3'–dideoxy–inosine and 2',3'–dideoxy–adenosine

9-[2(phosphono-methoxy)ethyl]-adenine, 9-[2(phosphono-methoxy)propyl]-guanine, 9-[2(phosphono-methoxy)propyl]-adenine for the treatment of HIV1 infections.

12. The therapeutical composition according to claim 11 for the treatment of AIDS related neoplasias

13. The therapeutical compositions according to claim 10 wherein said antiviral agents are selected from the group consisting of acyclovir, 9-[2(phosphono-methoxy)ethyl]-guanine and adenine-arobinoside.

14. A process for preparing the compound as claimed in claim in anyone of claims 1 or 2 wherein R = COOR', COOH, R^1 is = I, R^2 = CH_2OH, $CH_2OPO_3Na_2$, $CH_2OPO[OCH_2OCOC(CH_3)_3]_2$ or $CH_2OPO[OCH_2CH_2SCOC(CH_3)_3]_2$, $R^3 = R^4$= OH and n=0,

comprising the following steps:

a) reacting the intermediate of formula (I) wherein R, R^1 and n have the above mentioned meanings and $R^2 = CH_2$-OCOPh, $R^3 = R^4$ = OCOPh, with sodium methylate in the presence of methanol as the solvent, thereby obtaining the compound of formula (I) wherein R = $COOCH_3$, R^1 = I, $R^2 = CH_2OH$, $R^3 = R^4$ = OH and n = 0,

b) reacting the compound obtained in step (a) with an aqueous solution of sodium hydroxide thereby obtaining the compound of formula (I) having R = COOH. R^1 =I, $R^2 = CH_2OH$, $R^3 = R^4$ = OH and n = 0.

or

b') reacting the compound of formula (I) obtained in step (a), with terbutyldimethylsilylchloride in dimethylformamide thereby obtaining the compound of formula (I) wherein R= $COOCH_3$, R^1 =I, R^2= $CH_2OSi(CH_3)_2C(CH_3)_3$, $R^3 = R^4 = OSi(CH_3)_2C(CH_3)_3$, which compound is then treated with the alcohol R"OH, wherein R" is a C_2-C_5 alkyl radical in the presence of sodium hydride in benzene, thereby obtaining the compound of formula (I), wherein R is COOR", R^1 =I. R^2= $CH_2OSi(CH_3)_2C(CH_3)_3$, which compound is finally converted into the product of formula (I) wherein R= COOR", R^1 =I. $R^2 = CH_2OH$, $R^3 = R^4$ = OH and n = 0, with ammonium fluoride in methanol,

or

b") reacting the compound of formula (I) obtained in step (a) with $POCl_3$ and pyridine in a mixture of acetonitrile and water successively salififying the obtained product with cationic

exchange resins, thereby obtaining the compound of formula (I) wherein R = $COOCH_3$, R^1 = I, R^2 = $CH_2OPO_3Na_2$, R^3 = R^4 = OH and n = 0,

or

b''') reacting the compound of formula (I) obtained in step (a) with bis[(pivaloyloxy)methyl] hydrogen monophosphate in the presence of triphenilphosphine and diethyl azodicarboxylate in dimethylacetamide as the solvent, thereby obtaining the compound of formula (I) wherein R = $COOCH_3$, R^1 = I, R^2 = $CH_2OPO[OCH_2OCOC(CH_3)_3]_2$, R^3 = R^4 = OH and n = 0

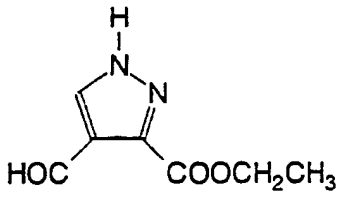
or

b^{iv}) reacting the compound of formula (I) obtained in step (a) with 2,2 dimethoxypropane in the presence of bis-(p-nitro-phenyl)-phosphate in acetone thereby obtaining the compound of formula (I), wherein R = $COOCH_3$, R^1 = I, R^2 = CH_2OH, R^3 and R^4 form an isopropylidene group and n= 0, which compound is then reacted with bis (S-pivaloyl-2-thioethyl)N,N-diisopropyl-phosphoramidite in tetrahydrofuran in the presence of 1-H tetrazole at room temperature and successively with terbutylhydroperoxide, thereby obtaining the compound of formula (I) wherein R = $COOCH_3$, R^1 = I, R^2 = $CH_2OPO[OCH_2CH_2SCOC(CH_3)_3]_2$, R^3 and R^4 form an isopropylidene group and n= 0, which compound is finally treated with p-toluesulfonic acid in methanol thereby obtaining the compound of formula (I) wherein R = $COOCH_3$, R^1 = I, R^2 = $CH_2OPO[OCH_2CH_2SCOC(CH_3)_3]_2$, R^3 = R^4 = OH and n = 0.

15. The process as claimed in 14 wherein in step (b) NaOH 0.035 M is used, in step (b''') the reaction temperature is 60° C.

16. The process for preparing the compounds as claimed in any one of claims 1 or 2 wherein R is $COOCH_3$, R^1 = C≡CSi$(CH)_3$, R^2 = CH_2-OCOPh, R^3 = R^4 = OCOPh and n = 0 , comprising reacting the compound of formula (I) wherein R^1 = I, R^2, R^3 and R^4 have the above mentioned meanings with trimethylsilylacetylene with a catalytic amount of bis(triphenylphosphine) palladium dichloride and CuI in triethylamine at a temperature ranging from 60 to 80°C.

17. A process for preparing the compound as claimed in any one of claims 1 and 2, having R = $COOCH_2CH_3$, R^1 is = CHO, CH=$CHNO_2$, CH=CHBr, CH=CH_2, CH=$CHCOOCH_2CH_3$, R_2 =CH_2OCOPh, R^3= R^4, OCOPh, n=0 comprising reacting the 4-formyl-3-ethoxycarbonyl pyrazole having the formula:



with hexamethyldisilazane with 1-O-acetyl-2,3,5-tri-O-benzoyl-ß-D-ribofuranose in the presence of trimethylsylylchloride and trifluoromethanesulfonate thereby obtaining the compound of formula (I) wherein R is $COOCH_2CH_3$, R^1 is =CHO, R^2 = CH_2OCOPh, R^3 and R^4 are OCOPh,

b) reacting the compound of formula (I) obtained in step (a) with nitromethane and aluminum oxide thereby obtaining the compound of formula (I) wherein R, R^2, R^3, R^4 and n have the above mentioned meanings and R^1 is $CHOH-CHNO_2$, which compound is in turn converted into the compound of formula (I) having R^1 = $CH=CHNO_2$ with mesyl chloride;

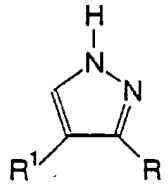
or

b') reacting the compound of formula (I) obtained in step (a) with a compound selected from the group consisting of: $Ph_3P=CHCOOCH_2CH_3$ and Ph_3PCH_2Y Br, wherein Y is H or Br thereby obtaining the compound of formula (I) wherein R, R^2, R^3, R^4 and n have the same meanings and R^1 is respectively $CH=CHCOOCH_2CH_3$, CH=CHY, wherein Y has the above mentioned meanings.

18. The process as claimed in claim 17 wherein, step (b') is carried out in the presence of n-butyllithium when Ph_3PCH_2Y Br is used as the reactant.

19. A process for preparing the compounds as claimed in any one of claims 1 and 2 wherein R is $COOCH_2CH_3$, R^1 is CHO, $-C\equiv C-Si(CH_3)_3$, $-C\equiv CH$, CHO, $CH=CHNO_2$, CH=CHBr, $CH=CH_2$, $CH=CHCOO_2CH_3$, R^2 =R^3 = R^4 = H, n=1, comprising the following steps:

reacting the compound of formula (II)



(II)

wherein R^1 is I, CHO with 2,3 dihydro–4–pyran in chloroform in the presence of paratoluensulphonic acid, thereby obtaining the compound of formula (I), wherein R^1 is I or CHO, R, R^2, R^3 and R^4 have the above mentioned meanings,

b) reacting the compound of formula (I) obtained in step (a) having R^1 = I, with trimethylsilylacetylene in the presence of bis(triphenilphosphine)palladium dichloride and CuI in triethylamine at temperatures ranging from 60 to 80°C, thereby obtaining the compound of formula (I) having R^1 = C≡C–Si$(CH_3)_3$, which compound is optionally treated with tetrabutylammoniunfluoride in tetrahydrofuran thereby obtaining the compound of formula (I) having R^1 = C≡CH,

or

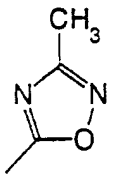
b') reacting the compound of formula (I) obtained in step (a) having R^1 = CHO with nitromethane and aluminum oxide thereby obtaining the compound of formula (I) wherein R, R^2, R^3, R^4 and n have the above mentioned meanings and R^1 is CHOH–$CHNO_2$, which compound is in turn converted into the compound of formula (I) having R^1 = CH=$CHNO_2$ with mesyl chloride,

or

b") reacting the compound of formula (I) obtained in step (a) having R^1 = CHO with a compound selected from the group consisting of: Ph_3P=$CHCOOCH_2CH_3$ and Ph_3PCH_2Y Br, wherein Y is H or Br thereby obtaining the compound of formula (I) wherein R, R^2, R^3, R^4 and n have the same meanings and R^1 is respectively CH=$CHCOOCH_2CH_3$, CH=CHY, wherein Y has the above mentioned meanings.

20. A process as claimed in claim 19 wherein, step (b') is carried out in the presence of n–butyllithium when Ph_3PCH_2Y Br is used as the reactant.

21. A process for preparing the compound as claimed in anyone of claims 1 and 2, for preparing the compound wherein R is:



R^1 is = I. R^2 =CH_2OH, R^3 = R^4= OH,

comprising reacting the compound of formula (I) wherein R =$COOCH_3$, R^1 = I, n=0 R^2 = CH_2-OCOPh, R^3 = R^4= OCOPh and n=0, with acetamidoxime in the presence of NaH in tetrahydrofuran.

22. 4-formyl-3-ethoxy-carbonyl-pyrazole.

23. A process for preparing the compound as claimed in claim 23 comprising reacting ethylpyruvate semicarbazone with $POCl_3$ in dimethylformamide.

INTERNATIONAL SEARCH REPORT

International Application No
PC., EP 96/02485

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07H19/04 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07H A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF MEDICINAL CHEMISTRY, vol. 35, 1992, WASHINGTON US, pages 917-924, XP002018485 MANFREDINI S. ET AL: "Pyrazole-Related Nucleosides. Synthesis and Anitviral/Antitumor Activity of Some Substituted Pyrazole and Pyrazolo[4,3-d]-1,2,3-triazin-4-one Nucleosides" cited in the application see the whole document	1-3,14

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 November 1996	22. 11. 96

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